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MORRIS B. BENDER: AN APPRECIATION

EDWIN A. WEINSTEIN, M.D.
Guest Editor

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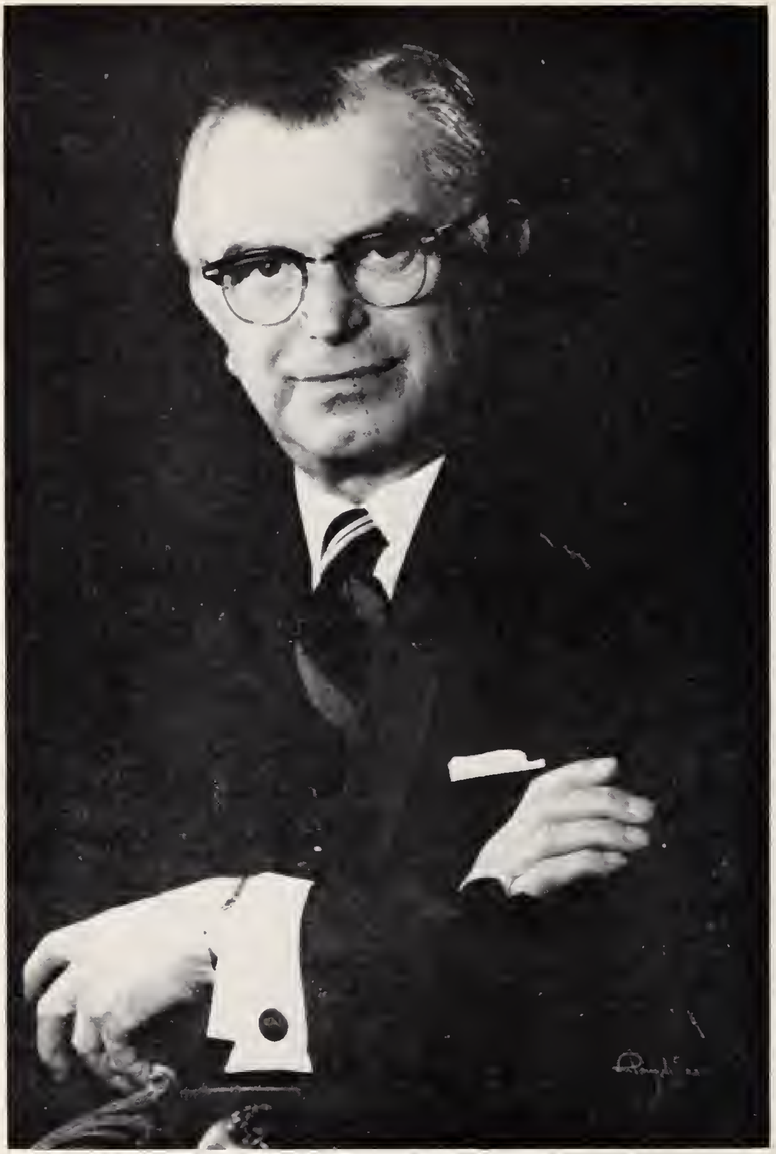

MORRIS B. BENDER, M.D.

Editor's Note

These papers by past and present colleagues of Dr. Morris B. Bender commemorating his service as Henry and Georgette Professor of Neurology and Chairman of the Department are in every sense a history of the growth of Neurology at Mount Sinai over the past forty years. The contributions on neurophysiology, neurochemistry, immunology, virology, and cellular physiology represent the development of these disciplines under his tenure. Other papers reflect his special personal interest in the areas of oculomotor function, perception and language, disorders of behavior associated with alterations in brain function, and the treatment of brain tumors and subdural hematomas. While each paper in the volume is the work of its author, most display some of the imaginativeness, ingenuity, and intellectual curiosity of MBB.

I think that I can express for the other contributors, as well as for myself, the pleasure it has been to offer this tribute to Morris Bender. A particularly happy thought is that his "retirement" is a mere technicality and that for years to come he will continue to confound, confuse, and contradict his colleagues.

*Edwin A. Weinstein, M.D.**

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* Dr. Weinstein, the Guest Editor, has been a long-time associate of Dr. Bender, working with him on the physiology of the brain stem and subsequently on disorders of perception and communication.

Morris B. Bender: An Appreciation

MELVIN D. YAHR, M.D.*

In 1975, the Department of Neurology will celebrate its 75th Anniversary. Its tremendous growth from twelve beds and four staff members to its current status with 52 beds, more than 65 staff members, and extensive research and diagnostic laboratory facilities has largely taken place during the regime of Morris B. Bender who, following 18 years of service as Adjunct and Associate Attending Neurologist, became Neurologist-in-Chief of The Mount Sinai Hospital in 1951 and Professor and Chairman of Neurology of the Mount Sinai School of Medicine in 1966. In dedicating this issue of *The Mount Sinai Journal of Medicine* to Morris Bender, his colleagues recognize not only his contributions to Mount Sinai but, even more importantly, his stature as a neurological scientist and educator who has played a major role in establishing and advancing our specialty the world over.

In this day and age, it is rare to stamp individuals as adherents of a particular school or philosophy of medical practice. Yet, a newcomer to Mount Sinai is impressed that a Benderian School of Neurology indeed exists. Its disciples can hardly be classed merely as loyal students; rather, they are an inquisitive group of investigators and clinicians with a sophisticated approach to the many and varied problems of the nervous system. My first encounter with this approach dates from a chance meeting with Mo in the early 1940's. I use the term "meeting" in its broadest sense, for I was a medical student at NYU and Mo an attending physician visiting the neurological service on leave from his Navy assignment. I well remember being impressed with and admiring his contribution to a case under discussion in which he demonstrated the phenomenology, recognizing with precision the significance of the clinical findings, their relationship to the disturbance of neural function and the type of underlying pathological process. Over the succeeding years, my associations with him were at meetings of the American Neurological Association or through his numerous contributions to the neurological literature. His fundamental papers on ocular dysfunction and disorders of perception, coupled with novel and imaginative ways of demonstrating neurological deficits in patients, have contributed greatly to our further understanding of the form and function of the central nervous system.

Morris Bender's masterly clinical judgment and devotion to his profession are legendary at Mount Sinai. They are only exceeded by the trust, admiration, and esteem in which he is held by his patients and the numerous colleagues who seek his counsel. Melded to this is the courage which he has in his own convictions. Often he has found himself in sharp conflict with those with opposing viewpoints. When he felt that others were beholden to dogma rather than fact, he had no hesitation in taking them to task. I recall the 1962 meeting of the American Neurological Association at which he presented a paper on the "Nonsurgical Management of Brain Tumors." Many in the audience shared his feelings but few were prepared to express them. The presentation earned for him the label of being anti-surgical, to mention one of the kinder adjectives. In perspective, he was neither pro nor anti any mode of therapy but rather he was presenting factually the results he had obtained in personally treating a large group of patients. The data were there and reported in true scientific fashion. If there was any bias, it was in favor of a considered concern for the patient, a characteristic he shared with his predecessor, Israel Wechsler. For indeed, Mo Bender has always been an advocate of the view once expressed by a wise physician who said, "The care of the patients begins with caring for the patient."

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* Dr. Yahr has succeeded Dr. Bender as the Henry P. and Georgette Goldschmidt Professor of Neurology and Chairman of the Department.

Over the years, neurological training has flourished at Mount Sinai. During Mo's tenure as Chief of Service, more than 200 neurologists have received their training under his tutelage. Many now hold important posts in medical schools and hospitals throughout the world. For his accomplishments, recognition has come from many quarters. Mount Sinai has honored him by awarding its Jacobi Medallion and the Gold-Headed Cane. American neurology has recognized his numerous contributions by electing him President of the American Neurological Association in 1973. With his retirement, he becomes the Henry P. and Georgette Goldschmidt Professor Emeritus of Neurology and Consultant to The Mount Sinai Hospital. His colleagues will continue to seek his wise counsel and advice as we attempt to carry on the Bender tradition.

Curriculum Vitae

Morris B. Bender, M.D.

Education

Central High School, Philadelphia, Pennsylvania, B.S.	1919–1923
University of Pennsylvania, Philadelphia, Pennsylvania, B.S.	1923–1927
University of Pennsylvania, Philadelphia, Pennsylvania, M.D.	1927–1931

Training

Intern, Temple University Hospital, Philadelphia	1931–1932
Assistant Resident in Neurology, Montefiore Hospital, New York	1932–1933
Resident in Neurology, Mount Sinai Hospital, New York	1933–1935
Assistant Alienist, Bellevue Psychiatric Hosp., New York	1935–1936
Assistant Psychiatrist, Kings County Mental Hygiene Clinic, Brooklyn	1/36–10/36

Postdoctoral Research

Honorary Research Fellow Yale University in Neurophysiology	1936–1938
Abrahamson Fellowship in Neuropsychiatry	1935–1938
Josiah Macy Jr. Foundation Fellowship	1939–1941 and 1947

Hospital and Medical School Appointments

Assistant Attending Neurologist, Mount Sinai Hospital, New York	1938–1946
Assistant in Neurology, New York University College of Medicine	1938–1939
Instructor in Neurology, New York University College of Medicine	1939–1942
Head, Laboratory of Experimental Neurology, New York University College of Medicine	1942–1950
Assistant Professor of Neurology, New York University College of Medicine	1942–1946
Associate Attending Neurologist, The Mount Sinai Hospital, New York	1946–1951
Associate Professor of Neurology, New York University College of Medicine	1946–1951
Senior Consultant Neurologist, US Veterans Administration, New York	1946–1961
Attending Neurologist and Chief of Service, The Mount Sinai Hospital, New York	1951–present
Director of Neurology, Bellevue Hospital (III Div.) New York	1951–1961
Professor of Clinical Neurology, New York University College of Medicine	1951–1966
Attending Neurologist, University Hospital, New York	1951–1961
Clinical Professor of Neurology, College of Physicians & Surgeons, Columbia University, New York	1951–1967
Consulting Neurologist, North Shore Hospital (Cornell University) Manhasset, New York	1953–present
Professor and Chairman, Department of Neurology, The Mount Sinai Hospital, New York	1967–1973
Henry P. & Georgette Goldschmidt Professor, Mount Sinai School of Medicine of The City University of New York	1968–1973

Memberships

American Neurological Association
American Academy of Neurology (Fellow)
American Psychiatric Association (Fellow)
American College of Physicians (Fellow)
American Physiological Society
American Psychological Association
Society for Experimental Biology and Medicine
American Association University Professors
American University Professors of Neurology
American Federation for Clinical Research
Association of Research in Nervous & Mental Diseases
Society for Biological Psychiatry
New York Society for Clinical Psychiatry
Harvey Society
Eastern EEG Society
American Medical Association
New York County Medical Society
New York Academy of Medicine (Chairman of Section, 1953–1954)
New York Neurological Society (President, 1961–1963)

Honors and Awards

Sigma Xi	1947
A.O.A. (Faculty)	1972
Jacobi Award, Associated Alumni of The Mount Sinai Hospital	1961
The Golden Cane Award	1971
Honorary Member, Northwest Neurological & Psychiatry Society	1962
Honorary Member, French Neurological Society	1970
Honorary Member, Israeli Neurological Association	1972
Honorary Member, Canadian Neurological Congress	1973
Honorary Member, British Neurological Association	1973
The Southern Cross of Brazil	1967
Treasurer, International Congress of Neurology	1965–1969
President-Elect, American Neurological Association	1971
President, American Neurological Association	1972–1973
United States Public Health Service, Special Consultant to Surgeon General (Visual Sciences)	1962–1964
Dr. Morris B. Bender Professorial Chair in Neurology	

Specialty Boards

American Board of Neurology & Psychiatry	1938
Workmens Compensation Board—SI 104394	1938
Mental Hygiene Board (Qualified Psychiatrist)	1939

Armed Forces

On Active Duty as Neurologist at US Naval Hospital, San Diego, California (Comdr., USNR)	1943–1946

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Morris B. Bender, M.D.

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14. Keschner, M., Bender, M. B., and Strauss, I.: Mental Symptoms Associated with Brain Tumor: A Study of 530 Verified Cases, JAMA 110:714–718, March, 1938.
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65. Teuber, H. L., and Bender, M. B.: The Significance of Changes in Pattern Vision following Occipital Lobe Injury, Am Psychologist 1:No. 7. 255-256, July, 1946. (Abst.)

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1948

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77. Bender, M. B., and Teuber, H. L.: Neuroopthalmology. *Progress in Neurology and Psychiatry for 1947*. Edited by E. A. Spiegel. Grune & Stratton, New York 1948, pp. 163–184.
78. Wortis, S. B., Bender, M. B., and Teuber, H. L.: The Significance of the Phenomenon of Extinction, J Nerv & Ment Dis 107:382–387, April, 1948.
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80. Weinstein, E. A., and Bender, M. B.: The Mid-Position Phenomenon in Eye Movements, Trans Am Neurol Assoc 73:163–165, June, 1948. (Abstr.)
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82. Bender, M. B., and Teuber, H. L.: Disorders in Visual Perception of Motion, Trans Am Neurol Assoc 73:191–193, June, 1948. (Abstr.)
83. Teuber, H. L., and Bender, M B.: Changes in Visual Perception of Flicker, Apparent Motion and Real Motion after Cerebral Lesion, Am Psychol 3:246–247, July, 1948. (Abstr.)
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1949

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The Syndrome of Lumbar Spinal Stenosis

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The significance of variations in the size and configuration of the spinal canal and their importance as causative factors in cord and nerve root compression have become increasingly apparent in recent years. Much of the earlier investigation was concerned with the cervical spine but a similar entity has been recognized in the lumbar region.

The stenotic lumbar canal syndrome initially described by Schlesinger and Taveras (1), has distinctive anatomic, clinical, radiologic, and therapeutic features. Its occurrence is more frequent than is commonly known, and, as a specific condition, it is often missed and neglected when evaluating lower spinal canal dysfunction. The syndrome shows no hereditary or systemic characteristics and has variable causes.

Anatomy

The lumbar spinal canal has a rather constant shape and a proportional volumetric size relative to the enclosed bony elements. The upper lumbar canal is more ovoid in shape, while the lower lumbar canal is triangular. When variations, either congenital or acquired, occur, the space available for the neural components in the canal and foramina becomes a critical feature.

In all forms of spinal stenosis, two abnormal features predominate. There is a reduction in the sagittal (ventral-dorsal) diameter of the canal and a constriction of the lateral recess where the emerging nerve root rests. A trifoliate configuration of the canal may then result.

Other architectural features may exist which make root compression more vulnerable. The neural arch, burdened by short and massive pedicles, thickened and vertically oriented laminae, and bulbous articular facets encroach upon the ventro-dorsal aspect of the canal and reduce the available space to a critical level where compression can occur. It is this available space which will determine whether or not discogenic disease, either protrusion or herniation or ultimately a bony ridge, will result in significant root compression.

Clinical

Patients present with features which suggest disc disease. There is often back pain which, when present, is never a predominant complaint. Sciatica, with accompanying positive neurologic findings, can occur. More frequent, however, is the presence of bilateral radicular pain with variable sensory, motor, and reflex deficits. Some patients present with symptoms suggesting intermittent claudication which subsequently proves to be unrelated to any peripheral vascular disease. The most dramatic presentation is that of paraparesis, areflexia, incontinence, and bladder distention, indicating cauda equina root compression. Less frequently, complaints of impotence and other autonomic nervous system dysfunction are elicited.

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Radiology

The failure to accurately evaluate the sagittal diameter of the lumbar spinal canal renders plain film diagnosis of this entity very difficult. However, the presence of bulbous facets approximating the midline, short pedicles and thickened laminae with resulting narrowing of the interlaminar space will indicate abnormal posterior components of the canal (Fig. 1A, B). Since routine films do not demonstrate the cross-sectional shape of the canal, nor indicate the degree of non-osseous changes, such as ligamentous and soft tissue thickening that may be present, myelography is needed for a definitive diagnosis.

The myelogram characteristically reveals posterior-lateral indentation of the thecal column at one or at multiple levels. Partial or complete blocks are seen, while postural changes such as flexion may reduce the degree of obstruction. Waist defects in the column are noted in the posterior-anterior view, whereas the lateral view shows a diminished ventro-dorsal diameter of the column (Fig. 2A, B). There may be accompanying disc and degenerative changes in the same region.

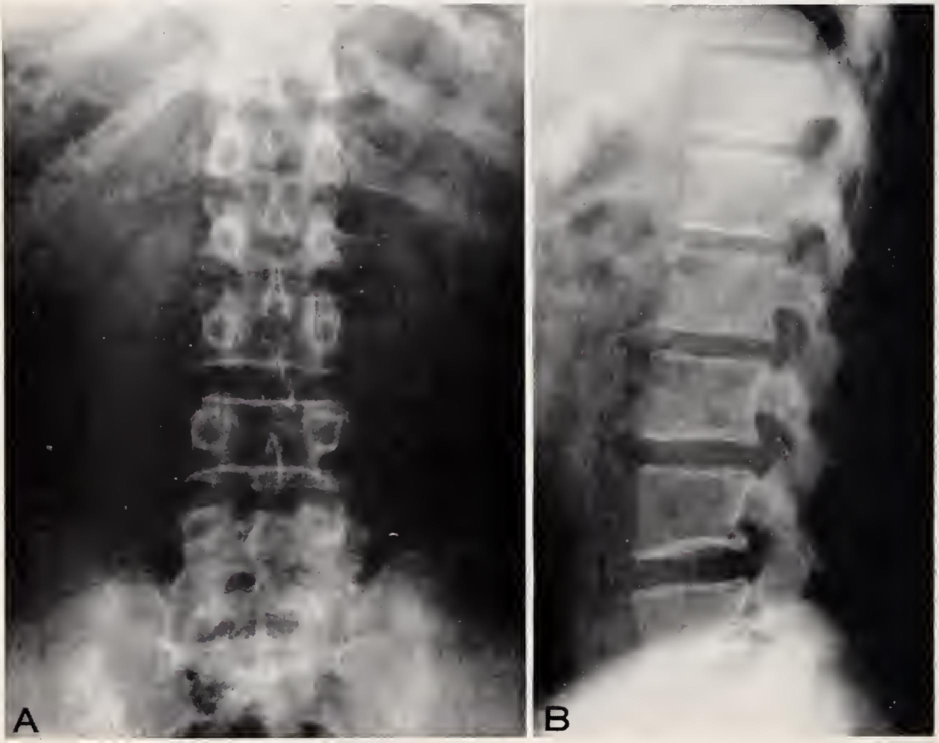

FIGURE 1

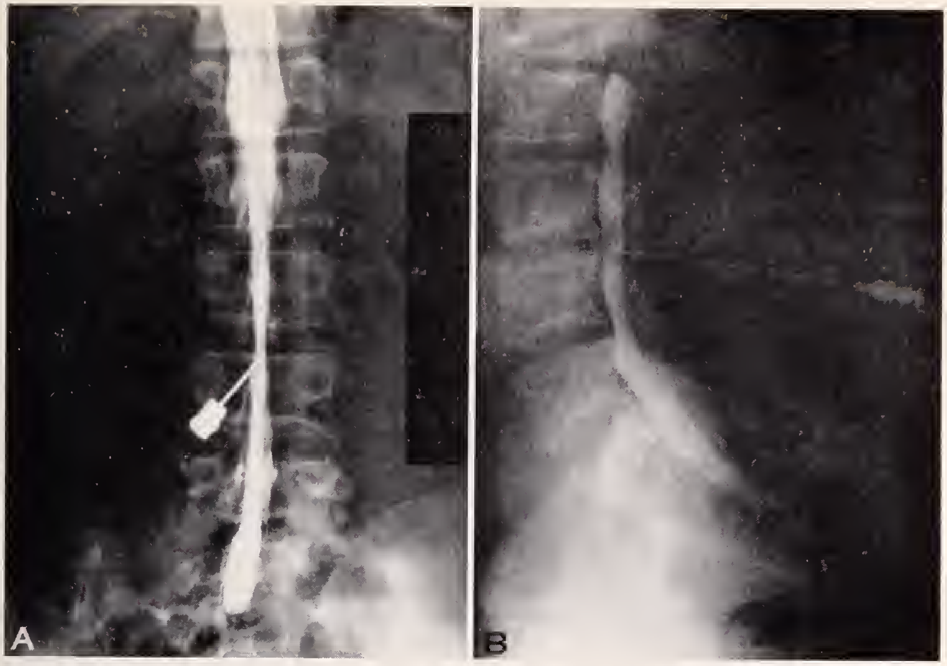

Figure 2

Etiology

The causes of lumbar spinal stenosis are either congenital or acquired. In congenital or developmental stenosis, the formative development of the lumbar spine is not sufficient in size to allow for adequate accommodation of the nerve roots and supportive elements. In the later decades, as intervertebral disc narrowing and degenerative changes occur, further compromise occurs and spondylotic narrowing (acquired spinal stenosis) results. Another form involves constriction of the canal in segmental fashion when either spondylolytic or degenerative spondylolisthesis is present. Achondroplasia and Paget's disease are less common causes of stenosis. Rarely, previous back surgery and fusion will segmentally constrict the canal.

Therapy

Conservative management with the intrathecal injection of steroids has provided some patients with relief of pain, but with only varying degrees of success in ameliorating their neurologic deficit. Since the compression of the roots in spinal stenosis is causing an entrapment neuropathy, surgical decompression may be necessary. The routine laminotomy or laminectomy, while sufficient for a localized disc herniation, is unsatisfactory when stenosis is present. Adequate and extensive laminectomy at several levels and foraminotomy, if indicated, are performed. Exploration of the disc space in the presence of spondylotic narrowing is therefore not indicated. In patients with back and lower extremity complaints, the results of therapy will largely depend on careful evaluation of the history, neurologic findings, plain films, and myelogram in each individual case.

Conclusion

The syndrome of lumbar spinal stenosis, with its varying clinical presentation, so often overlooked and mistaken for discogenic or degenerative spine disease, warrants consideration in the evaluation of this common problem. Significant improvement in the treatment of patients with back disorders can be anticipated when awareness and understanding of spinal stenosis exist.

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The Contribution of Morris B. Bender to Modern Concepts of Perception

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At the International Congress in Lisbon in 1953 I read a paper in German on "Visual Factors in Spatial Disorientation." Among the small audience was Morris B. Bender, patiently waiting for the end of my speech. Then he started discussion with the statement: "I didn't understand a word because I do not speak German. But I know your earlier writings on visual perception and would like to ask you some questions." This was the beginning of a long and rigorous cross examination which, finally, was stopped by the chairman.

This incident is more than a nice anecdote. It is in some way characteristic of Morris Bender's scientific attitude: An unpretentious curiosity with a meticulous search for facts rather than for complicated theories. Regarding scientific hypotheses, he is not afraid of unconventional ways of thinking, relying more on good common sense than on sophisticated speculations.

Before World War II Bender had demonstrated his interest in sensory defects by several papers on visual and tactile disorders, mostly in brain tumors. Then, his interest in problems of perception was stirred by the brain-injured soldiers of the war.

Gunshot wounds of the brain provide a unique material for the study of brain pathology. Contrary to brain diseases in peace time, mostly tumors and cerebrovascular accidents in old patients, missile wounds produce randomly localized, circumscribed lesions in an otherwise young and healthy brain. Therefore, this sad by-product of modern warfare has always stimulated brain research. World War I induced the work of Head in Great Britain, of Goldstein and Kleist in Germany.

During World War II Morris Bender, in a naval hospital in San Diego, had under his care a great number of patients with localized brain lesions from the Pacific theater. Unlike most of his European colleagues at that time, he could examine this vast material practically under peacetime conditions. Of this rare opportunity he made full use.

Together with a team of able collaborators whom he had inspired with his own scientific zeal, he systematically examined the influence of localized brain lesions on perceptual functions, especially in the tactile and visual sphere. After the war this research work was continued and completed by follow-up studies, checking of late sequelae, animal experiments, etc.

By these careful and persistent endeavors Bender and his associates eventually accumulated a wealth of empiric data which may cause envy to anybody who tries to do the same under less favorable conditions. The scientific value of this rich material is not only based on the great number of cases but, also, on a great variety of carefully devised and meticulously performed examinations testing all aspects of sensory perception from so-called "elementary sensations" to "higher gnosic functions." So, in addition, to the customary examination of simple touch, pin-prick, or temperature in the tactile field, there were tests on weight perception, extinction phenomena in double simultaneous stimulation, displacement of sensations, changes of body scheme, and similar disorders.

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* Dr. Bay of Düsseldorf has shared Dr. Bender's interest in disorders of perception and language for many years.

Even greater was the variety of tests in the visual sphere. Careful checking of visual acuity and of field defects under various standardized conditions were supplemented by tests for more complex visual phenomena, for instance, perception of motion, pattern vision, after-images, flicker fusion, visual adaptation, extinction and completion phenomena, and many others. The important role of eye movements for visual perception was carefully studied.

As could be predicted safely, such comprehensive and rigorous testing revealed a greater variety and a greater range of perceptual disorders, even in circumscribed brain lesions, than could be elicited with the limited number of comparatively crude tests used in traditional sensory examinations.

In patients where customary perimetry indicated only circumscribed visual field defects of a half field, a quadrant, or even a smaller scotoma, more sensitive methods of examination revealed broader areas of disordered function, while still other tests may have proved even a functional inferiority of the whole visual field. Such repercussion of a circumscribed field defect on the functioning of the whole visual system corresponds with other examples of interaction between different parts of the visual field, e.g., extinction and completion phenomena. In extinction, an object which is perceived in an impaired part of the visual field when exposed as an isolated stimulus, disappears when exposed simultaneously with a stimulus in the normal field. Completion means that an object, for instance, a circle, when exposed partially in the normal and partially in the hemianopic part of the visual field, is perceived as a full circle and not as a half circle (in the normal part of the field) as should be expected with point-to-point organization of the visual system. The same effect, namely perception of a full circle, is produced by exposure of only a half circle in the intact half field. An analogous phenomenon, studied intensely by Bender and his associates, is the perception of apparent movements across defective areas of the visual field. More recent papers have described the phenomena of palinopsia and palinacousis.

These observations have at least two neurophysiologic implications: a) They indicate that the conventional testing of visual field defects is not sufficient for a full evaluation of the functional state of the visual system. And, particularly, a circumscribed field defect (hemianopia or scotoma) does not permit the conclusion that the function in the residual field is "normal" and b) The dogma of a strict point-to-point correlation through the whole visual system from retina to striate cortex and further on to visual perception is only half of the truth. In addition to the evident correspondence between localized lesions of the cerebral substrate and circumscribed visual field defects there must be a functional interaction between different parts of the sensory field, or else phenomena as extinction or completion would be impossible.

Evidently, these facts have bearings on general theories about the formation and organization of sensory perceptions. In favorable contrast to renowned investigators in brain patholcgy with an enthusiasm for speculative theories, Morris Bender is, rather, anxious to back theoretical conclusions by hard empirical facts. But, feeling safe on solid experimental grounds, he is not afraid of unconventional ways of thought.

With such a scientific attitude and his rich empirical material it was inevitable that he soon fell afoul of old and venerable prejudices of brain pathology. This was especially true for the time-honored concept of agnosia as a specific disorder of "higher" perceptive functions in opposition to "elementary" sensory processes.

The clinical data of Morris Bender's group as well as of other investigators do not support the hypothetical differentiation of "elementary" versus "gnosic" sensory processes which could be distinguished and treated separately. Especially the experience that even in seemingly circumscribed field defects the functional impairment extends far beyond the border of the defect prevents the silent implication of unimpaired "elementary" functions in the residual field which, only, would justify the assumption of "gnosic" disorders. Bender's findings with his excellent methods of examination demonstrate that in lesions of the visual system the question of normal or impaired "elementary" functions largely depends on the accuracy of examination and on the sensitivity of the test methods employed.

Sufficiently detailed examinations such as those of Bender and his associates reveal "elementary" sensory defects and/or mental disorders as constituents of "agnosic" syndromes. Therefore, the dualistic structure of sensory perception with a lower level of elementary sensations and a superimposed level of higher "gnosic" perceptions can no longer be used as a model for the neurophysiologic organization of sensory processes.

The wealth of empiric data collected by Bender and his associates during and after the war clearly indicates that even the most localized damage to the visual system has far-reaching and diverse effects on many different sensory functions, exceeding simple deficits of one or another "elementary" sensory quality. Evidently, the neurophysiologic organization of perception is more complex than earlier investigators imagined and the simple—but unfortunately wrong—dualistic model suggested. For a better understanding of the complex neurophysiologic base of perception we need still more of such meticulous and unbiased examinations like those so masterly devised and performed by Bender.

The appreciation of Morris Bender's contribution to contemporary neuropsychology would be incomplete if we would confine it to these purely scientific aspects. By his own enthusiasm he inspired junior staff members at the San Diego Naval Hospital, at Bellevue, and at Mount Sinai in research work on the intriguing problems of perception. Many of these former juniors such as Hans-Lukas Teuber and William S. Battersby are now well-known seniors of neurophysiologic and neuropsychologic research. And—even more important—together with the interest for scientific work and for problems of perception, he inoculated his collaborators with rigorous standards for the reliability of experimental data and with a deep respect for the priority of solid empiric facts over theoretical constructs. Surveying the enormous output of contemporary neuropsychologic research, one wishes that these dominant features of Morris Bender's scientific attitude might have found a still wider propagation.

Useful Methods in the Diagnosis of Sellar Tumors

ANDREW L. BENDER, M.D.*

The syndrome of optic atrophy and bitemporal visual field defects with a normal sella turcica was described by Cushing (1) in 1930 and is now referred to as the chiasmal syndrome. Although it is taught that this syndrome occurs with tumors in the suprasellar area, particularly meningiomas, the chiasmal syndrome may occur in hydrocephalus where enlargement of the third ventricle causes the structures inferior to the optic recess to press on the chiasm (2). Other infrequent causes are multiple sclerosis (3) and vascular disease (4). In rare instances trauma (5) may cause the disruption of fibers in the chiasm producing the chiasmal syndrome. On the other hand, enlargement of a sellar tumor always results in pressure on neighboring structures and for this reason such neoplasms may present in a variety of ways.

Medical teaching notwithstanding, the practitioner has come to regard the chiasmal syndrome as pathognomonic of space-occupying lesions in not only the suprasellar but also the parasellar and retrosellar regions. This is a dangerous misconception because the chiasmal syndrome may not be manifested by even suprasellar tumors. Frequently, tumors in the sellar area present with enlargement of the blind spot, cecocentral and central scotomas, and headache. Infrequently, the patient may be aware that something is amiss with his vision, but this usually is not noticed until considerable central vision is lost. Schlezinger, Alpers, and Weiss (6) reported four cases of suprasellar meningioma associated with scotoma and headache. Symonds (7) discussed several cases in which the presenting sign of a sellar neoplasm was ophthalmoplegia. Weinberger, Adler, and Grant (8) discussed the syndrome of pituitary adenomas extending laterally to involve the cavernous sinus. Their series indicated involvement of the third nerve to be most frequent followed by the sixth and fourth nerves, in that order. When the fourth nerve was involved there was always involvement of another of the ocular motor nerves. The trigeminal nerve was never involved alone, even initially, unless one includes headache as a symptom, in which case the fifth nerve may be commonly involved early and alone.

Despite the varied symptomatology, many clinicians, unfortunately, have a rather rigid set of criteria for the diagnosis of sellar tumors. When one is not alert to the possibility of a sellar tumor, a misdiagnosis may occur with the unfortunate results which obtain by delays in definitive treatment. Two cases

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which demonstrate some common diagnostic pitfalls are discussed. Each patient presented with "nonclassic" visual symptoms.

Case 1: Mr. F.A., a 40-year-old longshoreman, had a history of frontal headaches which had increased in severity over the past year and had taken the patient to many physicians. Finally the patient noted some blurring of vision in his left eye. An ophthalmologist examined him, gave him tranquilizers, and told him to come back in a week. On the return visit he told the patient he had psychological problems. Two weeks later, vision in the left eye "closed up like a funnel." The patient sought hospitalization from another physician. There was a definite Gunn Pupillary Sign in the left eye. Visual field examination disclosed a very large, absolute, cecocentral scotoma in the left eye and a relative temporal field defect in the right eye. Angiography disclosed a large sellar tumor.

Comment

A scotoma frequently heralds the onset of a bitemporal visual field defect but in some cases a bitemporal field defect may never appear. Cushing and Walker (9) classified the visual field changes in hypophyseal tumors as bitemporal, homonymous, unilateral or bilateral amaurosis, and a fourth class which they called "bizarre." Most commendably, they were astute enough to note that some cases may have no visual symptoms. Traquair (10) points out that central scotomas are not seen in psychiatric problems; he makes the suggestion that uniform examinations be made at close intervals and that the results of these examinations be compared.

The Gunn Pupillary Sign may be easily elicited by the swinging flashlight test (11). For this test the patient is placed in a darkened room and told to fix on a distant object to minimize pupillary constriction from accommodation. A lit flashlight with good batteries is passed to and fro between the two eyes, pausing at each eye about five seconds. When the light is shining into the "good" eye both pupils constrict normally. When the light is then switched to shine into the defective eye the pupils will dilate somewhat. The flashlight should then be brought back to the "good" eye and the test repeated several times by passing the flashlight between the two eyes, hence the name "swinging flashlight test." During performance of the test the examiner should constantly observe the patient to insure that his gaze doesn't shift to the light itself, introducing pupillary constriction from accommodation. Sometimes it is best to hold the light so that it is just at the inferior margin of the orbit, out of the line of sight.

Even if a practitioner doesn't believe in the significance of the Gunn Pupillary Sign, when it is present, it at least suggests that additional tests are necessary. Careful examination of peripheral and central visual fields is often shunned or relegated to a technician because it is "too time-consuming." No further comment is necessary.

Case 2: Mr. V.F., an 18-year-old high school boy, was a mediocre student for most of his academic life. He began to complain of difficulties with vision; at first his vision appeared "cloudy"; an optometrist prescribed glasses. Sometime later, his vision deteriorated to the point where he saw so little he failed all his subjects in school. For some time he was given chiropractic treatment. He began to complain of seeing through a haze over which green and pink spots were floating; later he complained of metamorphopsia and seeing pink ants.

A physician was consulted and a diagnosis of conversion hysteria was made. Shortly thereafter, the boy was admitted to the hospital at the insistence of a relative, about two years after the onset of his visual problem. Vision was 6/120 OU. Skull films revealed an enlarged sella. Angiography confirmed the presence of a huge sellar tumor. At surgery both optic nerves were stretched to "string size." The patient lost all vision after surgery. He continued to see green and pink spots for several days but had no light perception in either eye.

Comment

This case is very unusual in that the patient had visual illusions, both formed and unformed, without direct involvement of the cerebrum by tumor. To the author's knowledge this has not been reported elsewhere.

"Cloudy vision" must be differentiated from "blurred vision." Cataract patients and patients with retinal or optic nerve disease describe "cloudiness." Simple refractive error usually manifests itself as blurring. A simple test for refractive error is the pinhole card. The patient whose vision is improved by looking through a pinhole card has at least some refractive error. In the presence of a clear optical system, if a pinhole does not improve vision then the disease process may involve the optic tract or retina. Metamorphopsia is occasionally seen in retinal disease but may be seen in occipital lobe or optic tract disease.

The differentiation of retinal disease from optic nerve disease is beyond the scope of this article. A gross test which may help the examiner is the photostress or retinal dazzle test. In this test the patient's visual acuity is measured in the standard way. The retina of the eye to be examined is bleached by exposure to direct, strong light. Within about thirty seconds, perhaps as long as a minute, the acuity returns to the pre-dazzle level in an eye with a normal retina. In those eyes with retinal disease, visual acuity may require several minutes or even longer to return to the pre-dazzle level. If a suitable instrument such as a Visuscope® or Euthyscope®* is available, it is possible to repeat the test with protection of the macula and/or with protection of the retina except for the macula.

Discussion

Two cases of sellar tumor presented with nonclassic findings. Had a careful assessment of optic nerve function been made, the presence of an anterior visual pathology would have been obvious. The common tests which are employed by the neurologist or ophthalmologist are quite selective but too insensitive to detect early disease of the optic nerve.

If the basic tests for optic nerve function fail to disclose an alteration in visual function in the face of complaints referable to the optic nerve then special tests should be carried out. Color discrimination with the Hardy-Rand-Rittler pseudoisochromatic plates is a sensitive test for disease in the optic pathway. The testing of each eye, individually, takes only a few minutes to perform. Scotometry, especially with the tangent screen at low light levels and with small colored targets frequently discloses defects that were unsuspected. The previously outlined swinging flashlight test is useful and probably should be done routinely in every neurologic examination.

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Once convinced that there is optic nerve disease the etiology must be established. The temptation to ascribe optic neuropathy to optic neuritis is a very strong one, but, in the face of continued deterioration of vision over a period of time, one's suspicion of other etiologic agents should be raised.

Skull films occasionally will demonstrate lesions in the sellar area but procedures such as tomography of the sella may be of help in cases in which the skull films are negative. Contrast studies should be performed in those cases with continued deterioration of vision without explanation. Frequently even an angiogram will not disclose a diagnosis until it is subtracted, or even subtracted and contrast-leveled.

Summary

Several simple methods of neuroophthalmologic examination helpful in the diagnosis of disease of the anterior optic pathway are presented. The application of two of these methods in two cases of sellar tumor with "nonclassic" symptoms is discussed. Patients presenting with subjective visual complaints can be screened rapidly for optic nerve disease with the methods presented. Those patients with objective optic nerve findings need further evaluation to determine the etiology of their disease.

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Oculomotor Hierarchy

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The theoretical concept (1) of a hierarchy of functions in the nervous system has been put to good clinical use by neurologists in recent years. For instance, by testing the sensory input to the visual system it was shown that movement is a stronger stimulus than shape which, in turn, is more effective than color (2).

A hierarchy for the motor loop of the ocular system has been suggested as well (2, 3) and constitutes the subject of this report.

Material and Method

Six normal subjects and four patients with disturbance of eye movements were investigated, full details of the technical procedure being reported elsewhere (4).

They were initially given various verbal commands such as "Look over there," then were tested for pursuit of objects, and finally oculocephalics with fixation were performed.

Eye movements were recorded by the usual E.O.G. technique and head movements according to the method of A. Atkin (5).

Results

In response to the command, "Look to the right" (Fig. 1), the eyes of a normal control execute a quick, single, rapid, large eye movement, followed at 20–30 msec by the head movement which is less ample and less rapid.

A patient with oculomotor disturbance (weakness in conjugate gaze secondary to a stroke) responds to the same command with an eye movement broken in "steps" of small amplitude and somewhat decreased speed as well as decreased amplitude (Fig. 2). Note, however, that the *relative* amplitude of the head is larger in the patient.

Testing pursuit of target (Fig. 3) shows again a somewhat "broken" pattern in the same patient with continuity of the eye movement, albeit of only fair quality.

Finally, oculocephalics with fixation is shown in Figure 4. This procedure is very well performed and the resulting record scarcely differs from that of a normal. These responses were characteristic of all patients tested.

Comment

Marquis (6) has shown, in mammals, various degrees of oculomotor dysfunction after occipital lobe lesions, thereby giving support to the concept of gradual acquisition of function.

Along these lines it is interesting to speculate that the gradual recovery of function in CNS seems to reduplicate the phylogeny process.

This concept gains further support from the examination of the oculomotor system in human pathology which shows the presence of various degrees of dysfunction at various times of recovery. Thus the eye movements in response to oral command are disturbed earliest in dysfunction of the oculomotor system. Target pursuit is better performed than movement on command and the oculocephalic response is similar to that of a normal subject. While our patients

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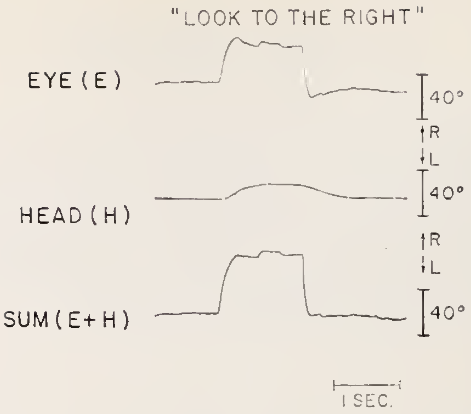

Fig. 1.

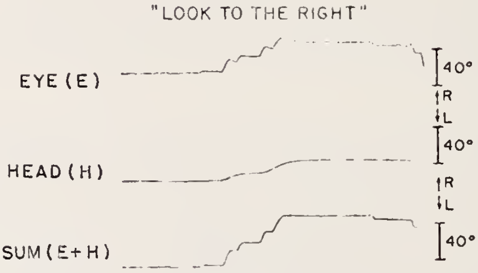

Fig. 2.

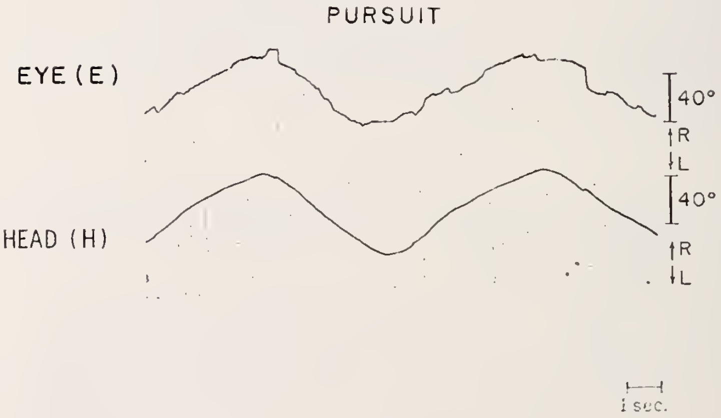

Fig. 3.

primarily were tested for eye dysfunction it became apparent that head movements were affected as well, but to a lesser extent.

The results underscore the importance of head contribution to the total eye and head movement in space (gaze), the head seemingly compensating for the defective eye movement. This is best shown by the eye movements resulting

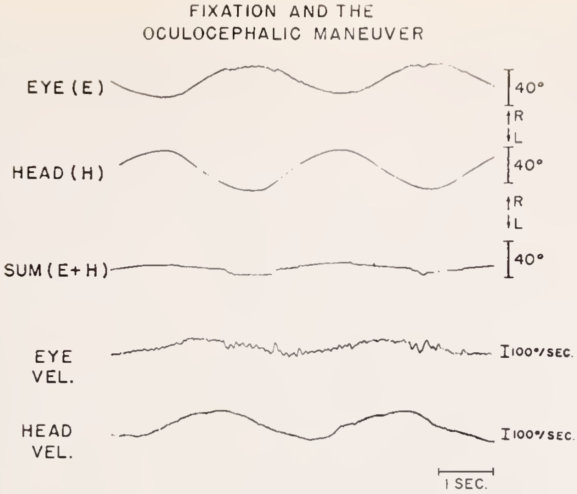

Fig. 4.

from oculocephalic maneuver which undoubtedly are secondary to head turning (5) and vestibular stimulation (7) and are close to normal.

Thus the clinical observations seem correct, and it appears likely that a hierarchy of function is present in the oculomotor system. The eye movements on command are last to recover (and earliest affected), followed by target pursuit. Last affected is the oculocephalic reflex. The eye and head are working as a closely connected system in most visual tasks and the greater the head contribution to the eye movement the less apparent will be the dysfunction of the latter in injury.

Acknowledgments

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On Dissociated Visual Functions

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Visual agnosia is commonly defined as a specific difficulty in the visual recognition of objects while the primary functions are intact (1). Often an anatomical division is postulated between primary and gnostic functions from ablation studies and from findings in brain-damaged patients. In many clinical cases a specifically visual agnosia is difficult to demonstrate because many patients show impairment of recognition and orientation in other sensory modalities as well. However, certain brain-damaged patients have relatively more difficulty in the visual than in other modalities. The fact of general mental impairment in many of these patients only establishes that if gnostic functions exist in the brain, either they are not modality-specific or else gnostic functions of different sensory modalities can be simultaneously injured.

The sine qua non of a discussion of gnosis is an experimental proof of primary functions. I do not believe that we have an adequate understanding of what primary functions are, even though we make use of them in clinical descriptions of visual malfunctions. In the accepted negative explanation, it is simply assumed that a patient's primary functions are intact if he has no change in visual acuity and no visual field defect. Visual field testing is based on the notion of spatial mapping of the retina on the cortex, and, on the whole, field defects after cortical ablation and in brain damaged patients have confirmed a retinotopical representation in the visual pathways. However, some clinical observations question the schema. G. Riddoch is credited with first observing that a dissociation of detection of moving versus stationary targets can occur in "blind" visual fields (2), and M. B. Bender and his colleagues, established that dissociations occur far more commonly than assumed. With the use of various stimuli they showed that a field defect is not necessarily absolute and its size or even "existence" depends on the type of stimuli used in the test. Dissociations can occur for colored, moving, or stationary targets, for flicker, or for bright-versus-dim targets in the "anopic" field (3). I will discuss our current research on two of the findings of Bender and his colleagues: a) "the so-called sparing of the macula is relative and visual function in this spared area is not entirely normal" (4); and b) "losses in sensitivity to flickering light can often be discovered in areas for which standard testing indicates a preservation of normal functions" (5).

Dissociation of Visual Acuity and Spatial Contrast Sensitivity

Classically, the smallest detectable test figure at 100% contrast on a background is a measure of visual acuity. Conversely, the minimum contrast neces-

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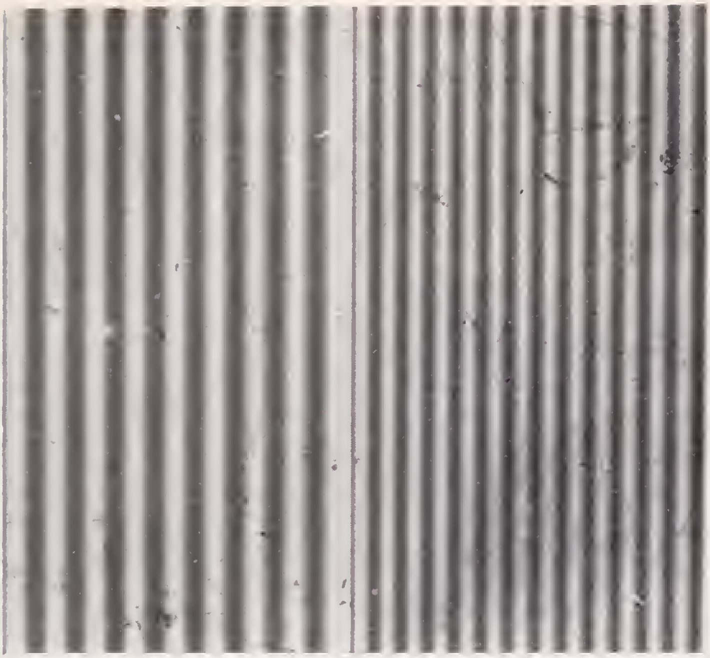

Fig. 1. Grating patterns of sinusoidal luminance profile of different spatial frequencies. From the viewing distance of 30 cm their spatial frequency will be of 1.3 cycles/degree (left) and 2 cycles/degree (right). The number of alternating bands in degree subtended visual angle is called the spatial frequency of the pattern.

sary to detect a stimulus on a background field depends on the size of a given stimulus. It is conventionally assumed that the larger the stimulus the less contrast required for its detection; even for the normal observer this is not necessarily so. This is revealed by spatial contrast sensitivity measurements which are tested by grating patterns as visual stimuli (Figs. 1 and 2). The threshold contrast necessary at each spatial frequency (see legend of Fig. 1) to detect the grating pattern instead of a homogeneous field establishes the contrast sensitivity of the observer (6). A plot of sensitivity of the normal observer at each spatial frequency yields the curve drawn with heavy lines in Figure 3. The last (extrapolated) point on the curve (indicated by an arrow) is the cutoff frequency which indicates the finest resolvable grating at 100% contrast. This corresponds to the visual acuity measured by conventional methods (7). We have established that in many patients with cerebral lesions who complain of blurred vision, the contrast sensitivity curve cannot be predicted by (simply) shifting the normal curve along the abscissa proportional to the reduction in visual acuity (8, 9). Data points in Fig. 3 represent the contrast sensitivity of a patient with a surgically proven meningioma pressing on the tip of the left calcarine cortex. His "conventional" acuity was 20/25. In order to evaluate his contrast sensitivity data a 'visuogram' was constructed (Fig. 4). This is a graph representing the ratio of the normal observer's contrast threshold to the con-

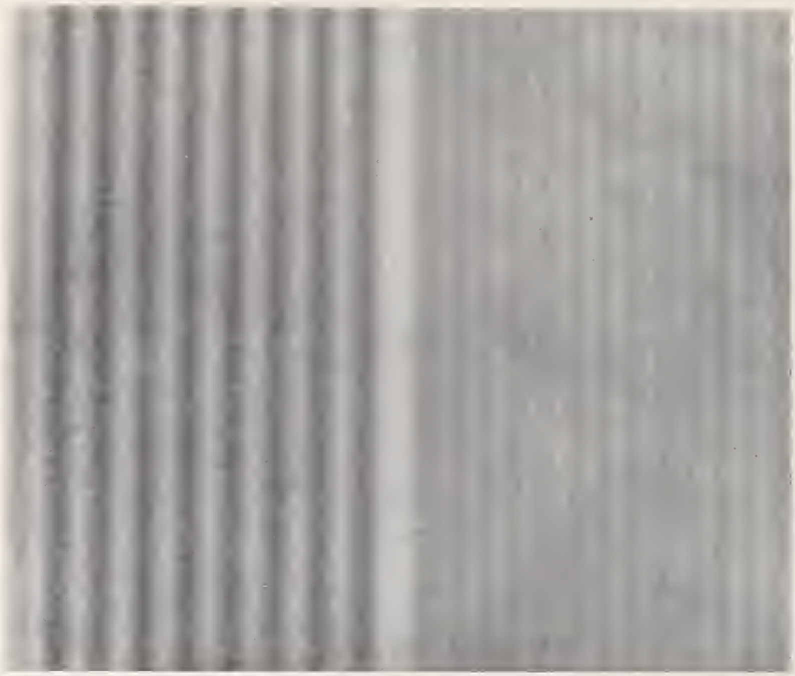

Fig. 2. The same grating patterns as in Fig. 1 with reduced contrast. Contrast is defined as the difference of the maximum and minimum luminance of adjacent bars over twice the sum of their luminances.

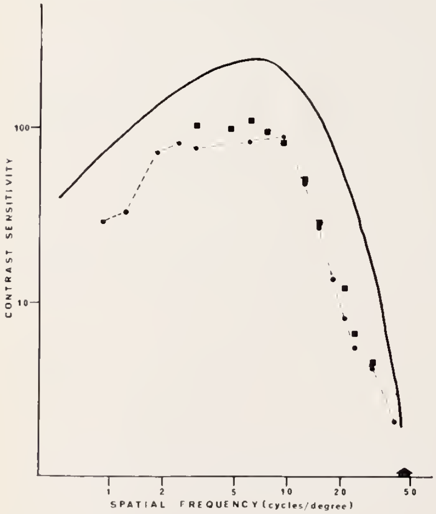

Fig. 3. The "normal" spatial contrast sensitivity curve (heavy lines) and contrast sensitivity data of a patient with cerebral lesion. Note the arrow; it points to the (extrapolated) cut-off frequency. This corresponds to the visual acuity measured by conventional methods.

trast threshold of a patient at equal bar sizes. In this particular case the visuogram revealed a mid-frequency dip suggesting a selective or channel loss in his visual system. In many patients the visuogram revealed disproportionately more loss at finer gratings (high frequency loss).

An explanation of this dissociation between visual acuity and contrast sensitivity emerges from electrophysiologic and sensory physiologic studies. Single cell recordings in the mammalian visual system revealed that cells have different receptive field sizes. Cells with different spatial frequency characteristics might be "looking" at the same retinal "point". A single retinal stimulus (say, a slit of light) will be optimal for one of these cells, but simultaneously many cells with different spatial frequency characteristics may respond. The energy contained in a slit of light is described by the "line spread function," which is analytically equivalent to the spatial frequency spectrum of the slit stimulus (6). Thus, if one were to calculate from cell responses the size of a stimulus which is projected on the retina, it could be done only from the simultaneous recording of many cells, each one responding to a different band of the spatial frequency spectrum of a stimulus (10, 11).

Evidence from sensory physiology (12) and evoked potential studies (13) suggests that cells with similar spatial frequency response characteristics are functionally grouped together in "channels" in the human visual system. Imagine the consequence of losing the use of some channels and not others. Most real visual stimuli are harmonically complex. If neural response to just one harmonic component of a stimulus is lacking, not even with a thorough (exhaustive) simultaneous recording of the remaining cells in the visual system could an experimenter reconstruct the exact retinal stimulus. The possible perceptual consequence of a "temporary channel loss" can be demonstrated with Fig. 5. From reading distance, fixate the center circle for about 30 seconds. Try to keep the eyes wandering along the circle's diameter in order to avoid conventional afterimages. Now after 30 seconds look back at this printed page. A peculiar distortion of the letter type of the text is noticed by many observers.

Dissociation of Spatial and Temporal Contrast Sensitivity

It is well known that in some patients flicker perimetry demonstrates visual dysfunction which cannot be demonstrated by conventional perimetric techniques (5). Is there any evidence of parallel organization of temporal and spatial functions in an intact visual system?

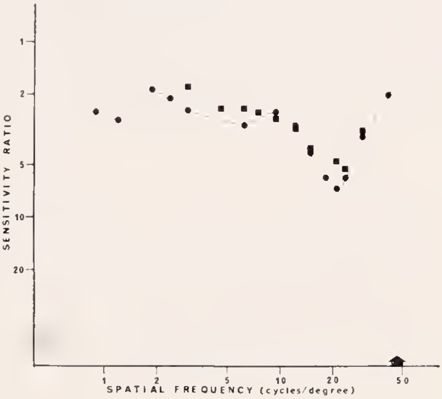

FIG. 4. Visuogram of patient whose data are presented in Fig. 3. The loss function (interrupted lines) is the ratio of the patient's contrast sensitivity to the normal contrast sensitivity at equal spatial frequencies.

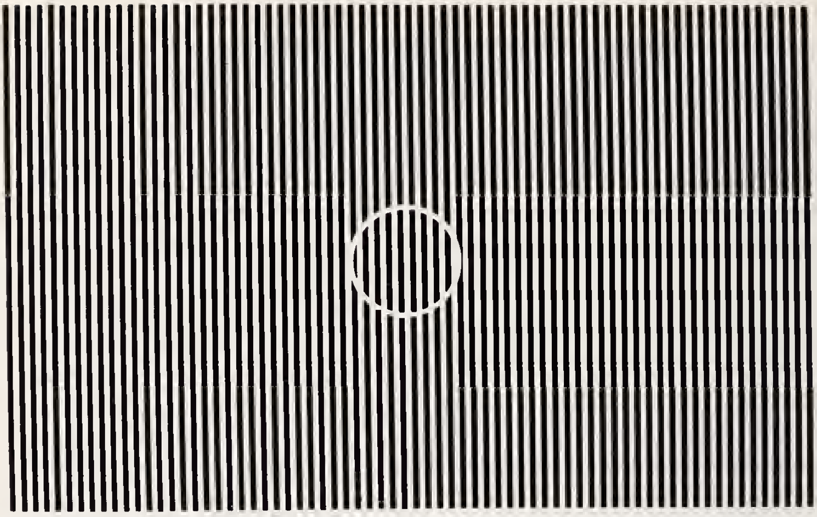

FIG. 5. A high-contrast grating pattern. From normal reading distance it falls in the mid-frequency region. Look at the circle at the middle for 30 seconds and then look at the printed text.

Recently we have developed the concept of "contrast modulation functions" in order to investigate spatio-temporal interactions in the human visual system. Contrast modulation consists of the successive presentation of a grating pattern at higher and lower contrast. It is as if the contrast of a grating pattern changed back and forth between, say, the contrast in Figure 1 and Figure 2 (see also Fig. 6). During the stimulus presentation, the modulation depth of a constantly visible grating pattern is varied. We call the contrast of the unmodulated (constantly visible) grating the "mean contrast." The observer is asked to indicate when he detects the minimum change in contrast. The minimum detectable change at different mean contrast levels establishes a contrast modulation function. Figure 7 presents experimentally obtained contrast modulation functions over a range of spatial frequencies (14). The data indicate that the slope of a contrast modulation becomes steeper with decreasing spatial frequency. In order to interpret, let us assume that a change is detected by cells "looking at" individual bars of a grating pattern (15). Then according to the slope found at low frequencies (ΔC = constant) cells which respond to the low frequency (coarse) gratings signal a fixed amount of temporal change irrespec-

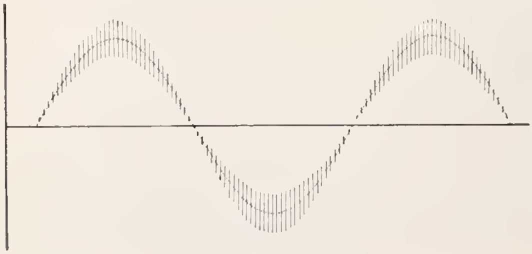

FIG. 6. Schematic illustration of contrast modulation. The contrast of a sinusoidal grating pattern changes between two contrast values. While one bar becomes brighter, its neighbor becomes darker, then the opposite occurs. Note that contrast modulation does not change the spatial and temporal average luminance of the pattern.

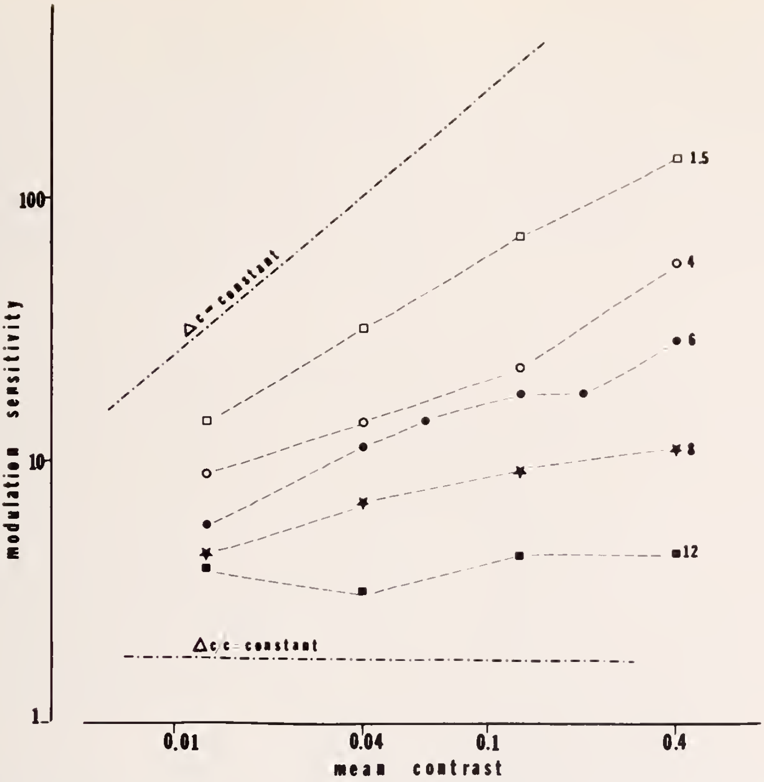

FIG. 7. Contrast modulation functions at different spatial frequencies. The abscissa represents mean contrast (C mean), the ordinate modulation sensitivity. Log/log scale. The number at the right of each function represents the spatial frequency of the pattern. The two interrupted lines indicate the limiting slopes of these functions. For ΔC/C mean = constant the slope is 0 (parallel line). For ΔC = constant the slope is 1 (45° line). Apparently in the explored range, contrast modulation functions fall between these two "laws" depending on the spatial frequency of the stimulus.

tive of the spatial contrast. The response to contrast changes of finer gratings cannot be explained by a cell "looking at" a single bar of the pattern. These responses are proportional to the *spatial* contrast ($\Delta C/\bar{C}$ = constant) (16). These results are compatible in the human visual system with the existence of "transient" and "sustained" detectors found in the cat's visual system (17). Our current experiments attempt to establish whether it is *necessary* to assume this organization.

Discussion

The study of any pathologic material presents the major problem of determining whether apparent fragmentations reveal a qualitative change (a fragmentation of a certain unity through the pathologic process) or whether they represent a quantitative change ("normally" present fragmentations more in evidence through the pathologic process). These two positions are not clearly considered in previous studies of visual functions in patients, but from some studies inferences can be drawn. Bay et al (18) were impressed by a certain constancy of various functions in the intact organism which is altered during the pathological change in the visual system. Whether their position represents qualitative or quantitative changes depends on an analytic definition of this constancy. Unless it can be defined in quantitative terms, the assumed constancy is an attribute of *quality*. Bender and his colleagues (19) felt that an "order of fragility" exists in the normal visual system. Thus their argument seems to be that functionally weighted functions of the normal visual system are *quantitatively* altered in patients.

To test spatial and temporal visual functions, we use stimuli which have been successfully employed in quantitative physiological studies of the visual system. Which position can we adopt concerning dissociated visual functions?

As far as spatial contrast detection in the intact visual system is concerned there is physiologic evidence that there is a "dissociation" of spatial functions (10, 13). In patients we tested, an "order of fragility" seems to be common, because we found that in general high frequency "channels" are more vulnerable to cerebral lesions than low frequency channels (8, 9). Further studies are required to establish whether the pathologically found functional weighting in spatial frequency discrimination is a property of the intact visual system.

There is evidence that dissociation of temporal and spatial discrimination occurs in patients (5, 20). Physiologic evidence of dissociation exists in cats, but a similar functional organization in the intact human visual system is not yet established. We are currently investigating this problem.

Dissociated functions undoubtedly require some kind of a division in some parts of the visual pathways, but at this point it is difficult to see whether the division is in the anatomic or chemical structure. Studying dissociated visual functions might be a promising area of conjoint physiologic and neurochemical investigations.

Studies of spatial and temporal contrast sensitivity functions allow us to investigate the cerebral organization of vision without an a priori assumption of a cartesian division of functions. Still, one must keep in mind that there is more to primary visual functions than "what the eye sees." Visual objects could be "memorized" by their significant spatial frequency components (21) and stored information could be used by the brain to match incoming information. Such a holographic-type process of visual detection could only be possible with a precise timing of this matching operation. Thus if responses to space and time are in parallel organization, spatio-temporal interaction might be considered as a secondary rather than primary function of the visual system. There is much to be learned about the organization of the brain from the study of dissociated primary visual functions.

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The Immunopathogenesis of Multiple Sclerosis*

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It seems particularly appropriate that a presentation of some new points of view about multiple sclerosis should appear in this volume dedicated to Dr. Bender. Dr. Bender's principle of creating an atmosphere of skepticism and challenge encouraged the growth of my own long-standing interests in research. The immediate impetus to investigate multiple sclerosis (MS) began while we were both standing at a patient's bedside wondering just what was going on in his nervous system. What process would produce a disease which was frequently expressed by transient but severe symptoms while the structural lesions; i.e., demyelination and sclerosis, were thought to be permanent and irreversible? The puzzle was further complicated by the poor correlation between the extent of involvement of the nervous system as revealed by the pathologist and the patient's clinical status as determined by the neurologist's evaluation. Usually, there were too many lesions for the amount of disability demonstrated by the patient. These "formes frustes" of MS may be thought to have reached their ultimate expression in two patients recently presented by Mackay and Hirano (1). At autopsy, these patients were noted to have widespread involvement of the CNS by sclerotic plaques although they had never been known to have experienced any neurologic difficulties during their lifetimes.

The inconsistencies and doubts which the classic techniques of gross and microscopic anatomy presented inspired little confidence that further applications of the same methods might offer significantly new data or ideas concerning the pathogenesis of MS. The possible application of tissue culture, as a living model system, seemed to offer at least a fresh approach to investigate these and other areas of neurobiologic and neuropathologic interest. With some assistance from the Dazian Foundation and a traineeship from the National Institute of Neurological Disease and Blindness, I was able to devote a few years to the development of a tissue culture technique under the tutelage of Mrs. Edith Peterson and Dr. Margaret Murray at the College of Physicians and Surgeons. Within a year, a method of culturing mammalian CNS had been established so that maturation in general and myelin formation in particular were encouraged to occur (2, 3). In addition, the reactions of the living tissue could be observed from day to day and photographed at will. These were thought to be significant

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since they added the dimension of time to the classic histologic examinations of fixed and stained tissues. Finally, in addition to myelin, the cultures developed interneuronal relationships, i.e., synapses, which were shown to propagate and transmit the nervous impulse and to respond to stimuli or to generate spontaneous complex bioelectric patterns representing polysynaptic organizations (4).

These organotypic cultures afforded a model system which could be used to test the hypothesis that MS resulted from an immunopathologic attack on the nervous system. This pathogenetic mechanism could also be simulated by a laboratory model involving animals, experimental allergic encephalomyelitis (EAE) (5–8). I thought that the in vitro system might be used to demonstrate antibodies in animals with EAE and patients with MS. Secondly, if antibodies were present, the reactions of the organized tissues might yield data concerning their significance in the disease process and, specifically, whether antibodies could produce, or reproduce, the anatomical lesions and neuronal dysfunction known to occur in vivo.

Our first series of experiments consisted simply of an attempt to demonstrate whether or not myelinated cultures of rat cerebellum would react in any way to serum from an EAE-affected animal. In this study, (9) rabbits were bled at various times following an inoculation with whole bovine CNS white matter in complete Freund's adjuvant (CFA). The serum was then added to the culture's nutrient medium fortified with fresh guinea pig serum as a source of complement. This mixture consistently produced a pattern of demyelination which, with light microscopy, was seen to begin within an hour or two after adding EAE serum in 25% concentration and was frequently complete in less than 48 hours. The response began as a swelling of neuroglia accompanied by swellings and distortions of the myelin sheaths. Later, the myelin was disrupted and broken into fragments. Finally, myelin fragments and apparently intact sheaths appeared to dissolve, leaving only fat droplets as evidence of their former presence. Silver impregnation of totally demyelinated cultures demonstrated that neurons and their axons were relatively unaffected. Normal rabbit serum and rabbit serum containing anti-rat kidney antibodies, prepared and kindly supplied by Dr. Beatrice Segal, produced no such effect on cultured rat cerebellum. The organ specificity of the response was substantiated in further experiments in which rabbits, guinea pigs, rats, and mice were exposed to bovine, guinea pig, or rat CNS tissue in CFA and their sera tested on cultured rat cerebellum or spinal cord or mouse cerebellum, spinal cord or cerebrum. The characteristic response was consistently present and always the same. Only rarely did a sample from an EAE-affected animal, inoculated with whole tissue in CFA, fail to demyelinate central axons. On the other hand, the myelin sheaths of axons originating from dorsal root ganglion cells, i.e., peripheral myelin, were totally unaffected by EAE serum in high concentrations. This observation of the organ specificity of the reaction has had its counterpart in studies of experimental allergic neuritis (EAN) in which animals are inoculated with peripheral nerve in CFA. Their sera and sensitized lymph node cells will demyelinate peripheral but not central axons (10–12).

After the specific pattern of demyelination in vitro had been firmly established, the cultures were exploited further to determine the immunologic characteristics of the active factors in EAE serum (13). It was demonstrated that complement was necessary for the reaction to occur, since its removal by exposure to heat, NH_4OH, or heparin destroyed the solution's demyelinating potency which could then be restored by the addition of complement in the form of fresh guinea pig serum.

Analysis of the serum revealed the activity to reside in the gamma$_2$-globulin (7S) fraction of rabbit serum. The albumin and alphaglobulin (19S) fractions were inactive. The myelinotoxic activity could also be removed by exposing the whole serum or its globulin fraction to rabbit or rat brain homogenates, whereas exposure to homologous or heterologous red blood cells, liver, lung or kidney had no such effect. These studies, therefore, provided the evidence that the myelinotoxic factors in EAE serum are antibodies.

In this same study, the localization of the serum fractions was examined by means of fluorescent antibody techniques. They revealed a specific localization of active globulins on the glial cell membranes and the myelin sheaths which, in culture as in vivo, are composed of modified glial cell membrane. As demyelination proceeded, a diffuse fluorescence was observed in the glial cytoplasm which contrasted sharply with the punctate pattern observed in control experiments with normal serum. In these, the globulin fractions did not concentrate at cell membranes, but seemed to have been incorporated into glial cells by pinocytosis, a function of these cells previously demonstrated by Klatzo and Miguel (14).

The next step was to examine samples of serum from patients with and without MS to determine whether or not they contained factors which would produce the same demyelinating response in culture. From our studies to date, it is clear that a majority (about 60%) of samples obtained from patients during the active stages of MS do, in fact, possess the same complement-dependent capacity to demyelinate cultures and that the pattern of the response is identical to that observed with EAE sera (15). Samples of serum which were obtained from patients during remissions or inactive stages of their illness rarely possess demyelinating potency. Control sera from normal subjects or from patients with destructive but not essentially demyelinative disease rarely produce an in vitro response. These data have been confirmed and extended by Lumsden (16). A striking, and unexplained, exception is the demyelinating activity of sera from patients with amyotrophic lateral sclerosis. This finding has also been confirmed (17) and will have to be studied further before its significance can be determined.

Classic neuropathologic concepts in the mid-twentieth century held that the mammalian CNS does not remyelinate. The living model offered by the cultured CNS tissues demonstrated, however, that remyelination would occur if the noxious factor, i.e., EAE or MS serum, were removed after an acute demyelinating experience and replaced with the tissue's normal nutrient (9, 15). At about the same time remyelination was demonstrated in the adult cat spinal cord previously subjected to a traumatic, demyelinating lesion (18). Subsequently, many investigators have reported remyelination in the mammalian CNS. Remyelination is not a rapid process, however. In the cultures, remyelination was observed to require weeks following the removal of myelinotoxic serum. In the cat, remyelination was observed some weeks to months after the traumatic episode. It may also be appropriate to note that remyelination in vitro and in vivo may be incomplete. That is, some axons may remain denuded. In addition, the thickness of the reformed sheaths may not attain that found in unaffected areas. When such remyelinated areas are examined after staining with Sudan black B, for example, they may have a gray rather than a black appearance and so may resemble a "shadow plaque." Thus, shadow or ghost plaques, previously thought to represent areas of partial demyelination may, in fact, result from incomplete remyelination. Evidence has now been advanced to suggest that the CNS of MS patients may also undergo some degree of remyelination (19, 20).

We have seen that remyelination will occur if the demyelinating antibody is removed from the culture's environment and replaced with normal nutrient. If, however, a relatively small amount of EAE serum; e.g., 1% by volume, is maintained in the nutrient, the tissue can be inhibited from remyelinating (21). This amount is usually incapable of demyelinating cultures. The inhibition will persist as long as the antiserum is maintained in the complement-enriched medium. In an interesting extension of this observation we added small amounts of EAE serum to the nutrient solution of 13–14 day mouse embryo spinal cord-dorsal root ganglion (DRG) cultures at the time of explantation and subsequently maintained them under its influence. The continuous presence of the antiserum in concentrations as low as 1% totally inhibited the differentiation of oligodendroglia and the formation of myelin. The inhibiting influence could be prolonged for at least 2 months. Peripheral myelination, i.e., around the axons of the DRG, was unaffected and proceeded in a normal fashion. The inhibiting effect of serum is also complement-dependent. The removal of the antiserum and its replacement with normal nutrient is promptly followed by differentiation of neuroglia and myelin formation even though a tissue may have been inhibited for as long as 60 days. These experiments are now being extended into an analysis of biochemical parameters of myelination.

The studies thus far have shown that immunologic factors present in EAE and MS can reproduce in culture an acute, demyelinating lesion resembling that of MS. Moreover, the cultures demonstrated, for the first time, that the mammalian CNS possesses the capacity to recover from this immunological attack and remyelinate when the aggressive antibody is removed from its environment. The next question asked was whether the immunopathologic factors could produce a state of sclerosis.

In this series of experiments (22) myelinated cultures were chronically exposed to relatively high (25%) concentrations of EAE serum. After varying periods of exposure to antiserum, e.g., 7, 14, and 21 days, groups of cultures were removed from its influence and returned to their normal in vitro environment. All cultures were examined daily for any changes in their appearance.

The expected pattern of demyelination was seen during the first 24 hours of exposure to EAE serum and the expected remyelination occurred when cultures were returned to normal in vitro conditions after a week of EAE influence. During the next 2 weeks, however, the cultures progressively lost their capacity to remyelinate. Few recovered after 14 days of exposure and, for all practical purposes, none after 21 days.

In addition, the cultured tissues were progressively transformed by long-term exposure to EAE serum. With the light microscope, one sees the tissue changed from its usual compact, epithelial character to a fibrous mass of cellular (glial) processes. At the same time, neuronal somata, whose cell boundaries are usually camouflaged or hidden by the surrounding neuroglia, now emerged from the background with unusual clarity. The image of the neurons against the altered background was thought to resemble eggs nestling among thick, rigid, intertwining twigs. These changes were also clearly seen in one micron sections. In the electron microscope, the process was observed to involve a gradually increasing accumulation of gliofibrillar material within astrocytes. Finally, their cytoplasm was almost entirely occupied by thick masses of gliofibrils with hardly enough space remaining for some mitochondria and glycogen granules. At the same time, the epithelial character of the tissue had obviously been destroyed. Instead of the usual cell membrane-to-membrane apposition with an intercellular space of about 150 Å, large, open spaces separated the glial and neuronal cell processes. This image, produced in culture, bears a striking resemblance to that observed in sclerotic plaques of MS and chronic EAE (20, 23).

The process of demyelination alone cannot be considered an adequate explanation for all the clinical phenomena in either MS or EAE. In the former, the rapidity of onset and recession of early symptoms, quite unrelated to their severity, is a challenge to search beyond the striking and dramatic fact of demyelination. Moreover, in the experimental model of EAE, entire species, particularly smaller rodents, may have few, if any, demyelinating lesions in spite of obvious clinical involvement. Even in groups of animals which do exhibit demyelination, the sequential examination of animals at intervals following inoculation with EAE-inducing suspensions reveals a disturbing percentage with clinically apparent disease but without any discernible CNS lesions (24, 25). Because of these apparent inconsistencies, we thought that the immunogenetic mechanisms might include antibodies which could directly affect the neuron and its function without necessarily implicating the neuroglial cell and myelin.

In the performance of these experiments, we (26) used long-term cultures of rodent cerebral neocortex and spinal cord which had developed myelin sheaths and synaptic junctions as well as a remarkable degree of physiological organization resembling complex networks of CNS in vivo. The characteristic response patterns of the CNS explants to electrical stimulation consist of two components: (1) simple spike potentials of brief duration, representing the propagation of impulses along axons and (2) complex potentials of much longer duration and latency indicating the generation of synaptic potentials in circuitous multineuronal pathways.

Exposure of the cultured cerebral and spinal cord explants to serum from animals with EAE (in concentrations of 10 to 25% by volume) produced extensive alterations in their bioelectrical properties long before any morphological changes had been detected. Electron microscopic studies of cultured spinal cord revealed no alteration in the typical synaptic ultrastructure after 1 hour of exposure to an EAE serum which produced functional block (as described below) within a few minutes in a simultaneously exposed sister culture. The bioelectric changes consisted of a disappearance of the complex responses characteristic of synaptic transmission within a few minutes after application of EAE serum. At this time, simple axon spikes could still be evoked. In some cases, the threshold for direct excitation of simple spikes also rose markedly, especially after prolonged exposure to serum. In the first studies of these reactions, serum samples from 6 rabbits with EAE were found to produce partial or complete block of complex bioelectrical response. Rabbit serum containing anti-kidney antibodies did not block bioelectric responses. Fresh serum from normal rabbits and guinea pigs were equally ineffective. The ability of potent EAE sera to block the transmission of the nervous impulse was also shown to be dependent upon the presence of complement.

When the effective serum was removed from the explant's environment and replaced with normal nutrient solution or balanced salt solution, the cultured tissue regained its usual bioelectric functions. Repeated or prolonged exposure to active serum decreased the rate and the extent of functional recovery of explants returned to control medium. At least partial recovery occurred in most experiments within 5 to 60 minutes.

Obviously, it then became important to determine whether or not serum obtained from MS patients during exacerbations of their illness possessed similar abilities to block neuronal and interneuronal function. In the first experiments, the sera of 2 MS patients were tested. Both, obtained during acute exacerbation, blocked interneuronal functions in exactly the same manner as did sera from animals with EAE. The human samples seemed less potent than some of the rabbit sera, requiring a 50 percent concentration to effect the same changes as 10 to 25 percent rabbit serum. In addition, a longer exposure was often required to attain the same degree of blocking the complex bioelectrical responses. Since that time, however, we have tested human (MS) sera which are as potent as many animal (EAE) samples. The blocking effect produced by human sera is also relatively rapidly reversed on removal of the serum and a return to control in vitro conditions. The serum from normal humans had no effect on the cultures. The inactivation of complement by heating the effective sera at 56°C for 20 to 30 minutes also destroyed their ability to alter neural function. The addition of fresh guinea pig serum as a source of complement restored the potency of heat inactivated serum. Confirmation of the presence of circulating, complement-dependent factors in MS sera capable of rapidly blocking polysynaptic activity has been obtained by Carels and Cerf (27) who employed a frog spinal cord preparation. In this instance, also, neuronal function returned relatively rapidly after removal of the offending serum.

In all of the above studies of the immunologically produced depression or abolition of polysynaptic interneuronal relationships and propagation of the nervous impulse, the dysfunction has been shown to be rapidly reversible. In MS and chronic EAE there is also ample evidence of recovery of function within relatively short periods of time following acute exacerbations. In time, however, neurological deficits become increasingly apparent and many patients become permanently and seriously affected. We attempted to reproduce this condition and have been able to observe a state of permanent functional deficit in cultures in which sclerosis has resulted from prolonged contact with demyelinating EAE serum. Even when these sclerosed cultures are washed free of the antiserum and returned to normal nutrient medium, they do not function normally. The first experiments (28) demonstrated that sclerosed cultures would respond to single stimuli with an apparently normal polysynaptic response. However, if they were placed under a mild stress, such as raising their temperature 1 to 2°F, (the tissue culture equivalent of the "hot bath" test) their bioelectrical activities were abolished. Returning the surrounding temperature to its normal levels produced a prompt return of function. Further explorations of the compromised functional capacities of sclerosed cultures are now in progress.

The direct comparison of serum from EAE-affected animals and MS patients by means of organotypic cultures demonstrates that both contain immunological factors which affect CNS tissues in identical ways. The antibodies have been shown to produce in vitro the pathognomonic lesions of MS, namely, demyelination with sparing of the axis cylinders and, eventually, sclerosis. The demonstration of similar immunological mechanisms in EAE and MS extends the previously observed histological similarity of lesions in animals and man and further validates EAE as a bona fide model for human demyelinating disease. Thus, the experiments support the hypothesis that the pathogenesis of MS involves immunologically-induced reactions.

These data have enlarged our understanding of the pathogenesis and pathophysiology of multiple sclerosis, suggesting that at least 2 groups of antibodies are involved; viz., anti-neuronal and anti-glial, and that there are a minimum of 3 stages in the response of the CNS to the antibodies; namely, primary neuronal dysfunction, demyelination and, finally, sclerosis. Of these, only the last seems to be irreversible.

The period of primary neuronal dysfunction in culture does not involve any discernible structural alteration. It is the result of a direct action of antibodies on the neuron which rapidly produce a depression or abolition of polysynaptic responses in the nerve network. Eventually, the propagation of the nervous impulse along the axon is similarly affected. The functional paralysis is rapidly reversible, disappearing within minutes to hours after removal of the antiserum. It is reasonable to suggest that anti-neuronal antibodies constitute a specific mechanism responsible for the transient symptoms of the MS patient. It is no longer necessary to invoke nonspecific responses, such as edema, to explain the temporary, though severe, neurological deficits in MS. Nor is it necessary to suggest that these fleeting disabilities are due to psychogenic rather than organic causes. The direct affect of antibody on the neuron may, therefore, be an early phenomenon in the disease process of MS. As a corollary to this hypothesis, one might expect to find a preponderance of anti-neuronal over anti-glial factors in patients during the early stages of MS. This would be particularly likely if the early exacerbations leave no residual neurological deficits. One recent serum sample seems to substantiate this possibility (Bornstein and Crain, unpublished data). It was obtained from a young man at the time when his second exacerbation of MS was receding, an attack which left no apparent residual dysfunction. The serum had no anti-glial activity; i.e. it produced neither demyelination nor inhibition of myelin formation. Yet, it contained a potent neuronal blocking action which rapidly suppressed conduction and transmission of the nervous impulse. Normal function returned shortly after removal of the antiserum.

Demyelination is a second stage of the neural response to an antibody's attack. These experiments indicate that almost all sera obtained from animals responding to an inoculation of whole brain tissue in CFA will demyelinate cultured mammalian CNS in the presence of complement. And, over 60% of the samples obtained from patients during an active stage of MS produce an identical response. Lumsden (16) reports an even higher incidence of demyelinating activity in MS patients. The mammalian CNS, however, can recover some if not all of its myelin after the immunological attack. Recent evidence suggests that the CNS of MS patients may also recover some of its myelin.

A permanent disability does, however, eventually occur in a large majority of MS patients. Even in those patients whose clinical status has apparently returned to normal, careful examination reveals significant amounts of neurological deficit, as Namerow's (29) studies indicate. The cultured material has shown that a prolonged application of demyelinating serum will produce irreversible structural changes which bear a striking similarity to sclerosis as seen in MS patients and animals with chronic EAE. This has been shown to be associated with a permanent state of functional deficit which persists even when the aggressive serum is removed. How dysfunction and sclerosis are related is not known. Neuroglia are usually classified into either oligodendroglia or astroglia. This simple division does not consider the possibility that they may serve many different and specific functions. These include myelin formation and maintenance, the transfer of nutrients from capillaries into the parenchyma, the nutrition of neuronal perikarya, the isolation of groups of synapses from others, etc., etc. One can speculate about the repercussions of reducing the neuroglial community to a single kind of cell, a fibrous astrocyte, whose cytoplasm is engorged with a mass of gliofibrils which hardly leave room for mitochondria and some glycogen granules. In addition, the intimate intercellular relationship, represented by the relatively small distances between cell membranes in the normal neuroepithelium, is largely destroyed in the sclerotic area and replaced by large extracellular spaces separating glia from neuron cell bodies and their processes. The reduction of glia to a uniform population and the physical disruption could irreversibly affect the economy of the community of cells and produce permanent dysfunction.

To summarize, by exploiting a particular tissue culture technique, we have obtained additional information in support of the hypothetical immunopathogenesis of multiple sclerosis. The immunological substances produce a gamut of responses in CNS tissue. These start with a functional neuronal blockade, which is not accompanied by any observable structural alteration and is relatively rapidly reversible. Then, patterned changes lead to demyelination while the structural integrity of the neuron remains largely intact. Demyelination is also reversible and some degree of remyelination is possible in the mammalian CNS. A final, probably irreversible, stage of sclerosis can be produced by prolonging the tissues' exposure to a potent demyelinating serum.

It must finally be stated, however, that we still don't know the answer to the first question, namely, details of the precise mechanisms which produce the loss of function manifested clinically by the MS patient.

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Medical Management of Intracranial Space-Occupying Lesions

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The prevailing practice in the management of neurologic patients is to divide them into medical and surgical and then to allow the neurosurgeon a free hand with the surgical patients. This is especially true for patients with intracranial space-occupying or mass lesions, which have traditionally fallen to the care of the surgeon. Referring physicians often consider the medical neurologist either as redundant or incompetent in the management of such patients, so that the neurologist may be bypassed when referral is made. There may have been good reason for this when surgery was the only feasible treatment and was often necessary for diagnosis, but many changes have occurred in recent years as will be described below.

In contrast, Doctor Bender has promoted the concept that the medical neurologist should be the principal manager in all neurologic cases and that the neurologist is in the best position to select diagnostic and therapeutic measures. He has further taught that therapy should not harm the patient and that harm is all too common with surgery. Because of these ideas, he and his school of neurologists have been considered antisurgical. However, those of us who have been influenced by him believe that we are selecting the best management for each patient. It might be expected that such a departure from traditional practice would be controversial and "Mount Sinai" or "Benderian" neurology often arouses strong emotions. It is understandable that neurosurgeons would be threatened by an invasion of their "turf"; however, some attacks have come from medical neurologists and this is harder to understand. Perhaps the reason is that many neurologists have not been trained to be able to handle "surgical" cases and are unwilling to take on the responsibility of patients with space-occupying lesions.

In this review, the management of intracranial space-occupying lesions will be considered with an emphasis on nonsurgical treatment of neoplasms and subdural hematomas (SDH) and the use of corticosteroids and osmotic agents for the treatment of cerebral edema. It is based on experience gained on Doctor Bender's service at The Mount Sinai Hospital. A general approach will be described with no attempt to cover the less common specific lesions.

The General Approach

After the discovery of an intracranial mass lesion, it is useful to first decide whether it is benign or malignant. Malignancy in the nervous system is a function both of location and growth potential. Lesions in the brain stem and

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deep midline structures of the cerebrum may be malignant by location since successful operative removal of such lesions is impossible. Even biopsies in such locations may cause unacceptable disabilities. Also multiple lesions and those spreading bilaterally, as through the corpus callosum, may be impossible to remove. Certain tumors, notably the malignant gliomas, have such a propensity for growth that they always recur no matter how completely they seem to have been extirpated at surgery.

It has been possible to avoid unnecessary operations in most malignant gliomas because modern neuroradiologic procedures—especially cerebral angiography—have provided reliable diagnostic criterion for malignancy. Such procedures can, in most instances, determine the precise location of lesions and nearly as often decide whether the lesion is intra- or extraparenchymal. An intraparenchymal lesion in an inaccessible location would never, except in the most unusual circumstances, be attacked surgically. On the other hand, most extraparenchymal lesions can be considered relatively benign and potentially amenable to surgery. The remaining intraparenchymal masses are then divided into vascular and avascular, depending upon whether the angiogram shows an increased vascularity of the lesion. This is commonly called a "stain." Since some benign lesions also show abnormal vascularity, it may be difficult to decide on the basis of this alone whether the lesion is malignant. Certain characteristics of the stain, such as arteriovenous shunting, exceptionally large and tortuous vessels, early draining veins, etc., when combined with an intraparenchymal site, are highly reliable in predicting malignancy. Multiple vascularized lesions also indicate malignancy due to metastases. Nonvascularized lesions, intraparenchymal in location, are more difficult to decide about, for although usually malignant, they occasionally turn out to be abscesses or hematomas. The former usually require surgery, while the latter respond well to medical treatment. Avascular lesions in general require exploration, particularly when their exact relation to the parenchyma is uncertain.

Once decision as to whether malignant or not has been made, one must then decide whether surgical treatment is likely to be beneficial. As in all forms of surgery, one must consider whether the mortality and morbidity is of such an order as to make the net result better than no therapy at all or than some alternative type of therapy. Published figures are not of much help in this matter because they are either inadequate or highly biased by a reluctance to report bad results. Some of the Mount Sinai experience has been published (1, 2).

Angiography

As a general rule, all intracranial masses should be evaluated by cerebral angiography. This is particularly true of masses at the base of the brain, such as in the region of the pituitary and chiasm, because giant saccular aneurysms occur in this region and must be distinguished from neoplasms. The angiogram is essential for deciding management; in particular whether a lesion is to be considered benign or malignant. A right brachial angiogram is the most benign method for investigation of supratentorial lesions and should be the initial examination in all suspected right-sided lesions and in poor risk patients with left-sided lesions. A left carotid angiogram is preferred in left cerebral lesions in patients who are not likely to suffer complications of carotid puncture and angiography—that is, those who are young and do not have vascular disease, as well as those higher risk patients in whom the right brachial angiogram has failed to provide sufficient information. Bilateral brachial angiography is essential to the radiographic diagnosis of all posterior fossa lesions and in some posteriorly located and basal lesions as well. Pneumocephalography may occasionally be a necessary supplement for accurate diagnosis, but ventriculography is hardly ever used.

Brain Scanning

The brain scan is primarily a screening examination. It does not ordinarily give information that would permit classification as benign or malignant, nor can it reliably tell us whether a lesion is intra- or extraparenchymal. When it shows multiple lesions, it may be possible to decide that one is dealing with metastases. The scan appearance of subdural hematoma is nearly pathognomonic, so that when the clinical picture is consistent a subdural hematoma may be diagnosed with confidence if the scan shows a typical "crescent" sign. We have encountered, however, one patient who had this sign, but whose angiogram was normal. The scan is less reliable in posterior fossa tumors and is most often positive in tumors of the hemispheres. Negative scans in the presence of angiographically proven masses are fairly common. False positives are not as much of a problem.

Temporization

With lesions of the nervous system, an attitude of "wait and see" is often the wisest. Today patients come to attention earlier than they once did, so that clinical and radiographic localization are difficult and may be inexact in early stages. Surgery at this time may result in "negative exploration." Occasionally a mass will result from such nonneoplastic conditions as infarction, demyelination, or inflammation; and follow-up studies will show that the mass is no longer present—the so-called "disappearing tumor." In other cases the mass may have grown and developed vascular and other characteristics of malignancy. In these situations, as well as in many others, periods of observation will prevent unnecessary operation. In malignant lesions, there is little to be gained by rushing to surgery and in benign lesions little is lost by waiting. An exception might be in those lesions of such size and accompanied by so much edema that there is danger of fatal herniation. As will be seen later, even in these cases, time can be bought with the judicious use of corticosteroids and osmotic agents.

Furthermore, a review of the general medical aspects of a patient may reveal such things as primary cancer elsewhere in the body, especially when appropriate laboratory examinations are employed. The value of extensive gastrointestinal radiographic procedures is doubtful, expensive, and often traumatic; but a radiograph of the chest and an abdominal radiograph immediately after the angiogram (this gives a satisfactory excretory urogram) are simple, inexpensive, and worthwhile and they should be done in all suspected central nervous system mass lesions.

Certain extraaxial or extraparenchymal tumors, although biologically benign, are situated so close to vital CNS structures that their removal often results in severe disability or death. These include large acoustic neuromas and other cerebellopontine angle tumors, meningiomas, chordomas, craniopharyngiomas, etc., which are ventral to the brain stem and hypothalamus. Such lesions are worth a period of observation. In some cases, the symptoms may remit either spontaneously, after a period of corticosteroid therapy, or after radiotherapy. In the case of pituitary adenomas, although surgical complications are fewer than in other lesions at the base of the brain, radiotherapy is so effective that it should be given a trial before surgery is considered.

Cerebral Edema

The brain responds with some degree of swelling whenever it is injured. Intracranial masses are nearly always accompanied by edema and often this is of such magnitude that it causes more dysfunction than the primary lesion. It is customary at autopsy in cases of cerebral glioma or metastasis to see a great amount of edema in addition to the tumor. This has also been demonstrated clinically when patients with such lesions are treated with corticosteroids and serial angiography shows a decrease in vascular displacement while the tumor as judged by abnormal vasculature is unchanged or even grows larger. Similarly, we have shown in patients with SDH treated with corticosteroids a decrease in the shift of midline vessels while the subdural avascular space was unchanged. Both in neoplasm and SDH the reduction in edema usually leads to improvement and often to complete recovery of function. Brain edema is most troublesome following cranial trauma and with cerebral neoplasm. However, edema may be a factor in vascular, inflammatory and toxic-metabolic disorders as well. This is particularly so in acute lesions.

Edema in the nervous system is, as elsewhere, an accumulation of fluid. However, because of the unique structure of the supporting cells in the nervous system, there are special features to the edema. For this reason, cerebral edema has been divided into two types (3). First is the cytotoxic type, in which there is a breakdown in cellular osmoregulation resulting in *intra*cellular swelling, particularly of the glia. The second type of edema is termed vasogenic, in which there is increased vascular permeability or a breakdown in the blood-brain barrier. In this type, there is *inter*cellular fluid accumulation. In clinical situations probably both mechanisms operate. Of the two principal methods of treatment of cerebral edema, the osmotic agents would be most effective in the cytotoxic type of edema, whereas the corticosteroids are believed to restore vascular integrity in vasogenic type of edema.

Studies of experimentally produced edema in various animals have shown varying biochemical and ultrastructural effects from treatment with corticosteroids or osmotic agents (4, 5); however, intracranial pressure is always reduced (6). Thus, it has been suggested that perhaps these agents caused shrinkage of the remaining normal brain, but then one would expect an increased displacement of vessels. This is certainly not true in humans as shown by our studies mentioned above. On the contrary, vascular displacement can be reversed by treatment with corticosteroids and osmotic agents. Although much remains to be learned about cerebral edema and the effects of steroid and osmotherapy, there is no doubt about the clinical effectiveness of these agents (7, 8) and future research should lead to more rational therapy.

Methods of Treatment

In clinical practice corticosteroids may be used to treat cerebral edema in acute cases in the form of dexamethasone given parenterally in an initial dose of 10 to 16 mg followed by 4 mg/6 hrs. It is doubtful that larger doses are of any benefit while smaller doses may be effective. For maintenance or in chronic cases, half the above amount of dexamethasone or prednisone 60 mg/24 hr is usually sufficient. When prolonged treatment is required the lowest effective dose is determined by gradual reduction 5 mg/week but no lower than 40 mg prednisone/day, below which level there is rarely satisfactory control of edema. Occasionally daily doses of up to 100 mg prednisone in the initial treatment of tumor or SDH or for maintenance are necessary. At such high levels side effects are apt to be excessive; however, it has been our impression that brain tumor patients have tolerated steroid therapy remarkably well and with a minimum amount of hypercorticism. Antacids are commonly given with the steroids to prevent peptic ulcer but it is doubtful that they have any effect in patients predisposed to ulcer formation. Such patients should generally not receive corticosteroids under any circumstances. The usual precautions and contraindications should be observed in steroid therapy of brain masses. We have found that diabetics can be so treated with little difficulty.

Osmotherapy has generally been reserved for acute situations because of its rapid action within minutes, while corticosteroids may take several hours and even longer for peak effect. Currently intravenous mannitol is most often used. It has the advantage over urea of not entering the cells of the brain and thus would be expected to give less rebound when discontinued. When a maximum and rapid effect is desired, mannitol should be administered intravenously in a 20% solution in 5% glucose, 500 ml (100 gm) within 30–60 minutes. Following this there must be maintenance therapy either with corticosteroids as described above or with mannitol continued in a dose of 200 to 300 gm/24 hr distributed throughout the maintenance I.V. fluids required by the patient. Often both steroid and osmotherapy will be utilized. Therapy for edema must be continued until its cause has in some way been relieved, otherwise there will be rebound and rapid deterioration. This is true both of acute lesions such as trauma and with neoplasms on long-term therapy.

At times long-term osmotherapy may be desirable either because corticosteroids can't be given or because it is more effective. Oral glycerol is useful in such cases. It is inexpensive and easily obtainable. In spite of its intense sweetness most patients are able to swallow it. The dose is 30 to 60 ml four times a day to give an effect equivalent to about 150 to 300 gm intravenous mannitol.

At the present time we would begin anti-edema therapy in any suspected intracranial mass lesion even before the diagnosis has been firmly established. In patients with papilledema, stupor, and rapidly developing symptoms such therapy should be intensive. With far-advanced cases, coma may be relieved and valuable time obtained so that a precise diagnosis and localization can be obtained and any required therapy can be properly planned and carefully done. It must be realized that medical measures cannot always control cerebral edema. In such cases there should be no hesitation to call on the surgeon—especially when there is any likelihood that a benign or reversible disorder is the cause.

In malignant or metastatic tumors with a good response to steroids, there is probably nothing to be gained from other types of treatment and such patients should be discharged and returned to normal activities. When no longer responsive to steroids, then radiotherapy and chemotherapy may be considered. Also, when the effect of steroids is not sufficient to restore good function, radio- or chemotherapy may be given sooner. Once started on steroid therapy, there is little chance that it can be discontinued. There is some suggestive experimental evidence that the glucosteroids have an oncolytic effect on gliomas. The many long survivals of patients with malignant glioma on steroid therapy also suggest some such effect.

Subdural Hematoma

The subdural hematoma is an extraparenchymal avascular mass which, although not a neoplasm, may grow so large as to be lethal from its mass effect. Such lesions were always considered surgical. In 1958 Doctor Bender encountered a patient with subdural hematoma who had only trivial symptoms. He was observed to show complete and lasting clinical and angiographic resolution without any treatment other than a few weeks in bed. Subsequently he attempted nonsurgical therapy in all subdural hematoma patients who were in good condition and found that some 40 percent would recover with only bed rest. In 1965 he began to treat such patients with corticosteroids. This made it possible to treat more advanced cases and increased the percentage of successes to 60%. Recently the addition of osmotic therapy promises to increase this figure even more. He has more than 100 patients who have been treated without surgery.

Probably none of his many innovations has caused so much consternation amongst neurologists and neurosurgeons. Following his earliest publication on the subject (9, 10) he was advised that it was "morally bad not to treat surgically" (11) and later, after publication of the electroencephalographic findings in his patients (12), an accusation of "brinksmanship" was made (13). An analysis of his cases shows that the patients do just as well as with surgery and that the avoidance of the surgical risk may give them a slight edge (14). His work has been confirmed by the Japanese neurosurgeons, Suzuki and Takaku, who successfully treated 22 of 23 successive patients with subdural hematoma with intravenous mannitol alone, with results they considered better than they had previously achieved with surgery (15).

Currently recommended management of subdural hematoma is to begin corticosteroid therapy as soon as the diagnosis is suspected. Osmotic therapy should be added in those who do not respond, who have had rapid progression of symptoms, or who are seriously ill with stupor or coma. The diagnosis should then be confirmed by angiography. Should the lesion be a small one with little vascular displacement and few symptoms, then the steroid medication can be tapered and stopped within a week. With larger lesions the steroids should be continued for two to three weeks depending on how rapidly the clinical picture improves. An occasional relapse may occur requiring retreatment. Comatose patients who do not respond within minutes to medical therapy and others who seem to be progressing in spite of such therapy should be sent to surgery without delay.

Summary

The "Benderian" approach to the management of intracranial space-occupying lesions would have the neurologist responsible for diagnosis and selection of therapy. This requires a sound knowledge of the types of lesions that occur, their radiographic appearance, and the best method of therapy—nothing, corticosteroids, osmotherapy, radiotherapy, chemotherapy, surgery, or other. Surgery is rarely worthwhile in malignant brain tumors and is extremely risky in certain benign tumors. Most subdural hematomas can be treated medically. We must continue to remind ourselves "to help, or at least to do no harm."

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A New Viral-Hematologic Etiology Hypothesis for Chronic and Paroxysmal Headache Syndromes

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The cause of the vast majority of chronic headaches remains obscure. While many headaches are regarded as "psychogenic" and patients are advised to seek psychiatric help or "learn to live with it," such advice affords little satisfaction either to patient or doctor. In my experience, psychotherapy, while it may benefit the patients' emotional problems, usually does not cure their headaches, and it is necessary to look beyond the term "tension headaches" to provide a heuristic and effective approach to the problem.

I am proposing a possible viral-hematologic etiology for a large number of the chronic, recurrent headache syndromes. I first considered this possibility when I noted that a number of patients with known herpes infection, particularly those involving the head, were prone to recurrent attacks of postherpetic neuralgia, without any overt evidence of activation of the basic lesion. Second, patients with trigeminal neuralgia who were treated with surgical rhizotomy had herpes lesions with a recurrence of attacks of facial pain. I then found that the incidence of cold sores, fever blisters, canker sores, and warts was definitely greater in patients with a background of chronic, recurrent headaches than in the general population of neurologic patients. I have now seen four patients who periodically get excruciating attacks of head pain only at the time they have the actual herpes lesions on their lips.

I would like also to reveal some personal medical history. I suffer from chronic recurrent headaches associated with my sinuses and I suffer from cold sores, really fever blisters, always occurring on the right side of my lips. I also suffer from a herpes progenitalia on the right, and, at times, I get a burning pain or streaking, shooting, sharp pains, either in my head or along the right lower extremity. However, these attacks can obviously occur in the absence of herpetic lesions.

My hypothesis is this. Virus and virus-like particles of the herpes or herpes-like groups exist in pain-sensitive structures. Under certain conditions, disease states or psychological stress, these neurotropic viruses located in the meninges and extracranial and intracranial vessels, can be activated in such a manner as to affect the platelets causing hyperaggregation and/or an alteration in platelet form which may result in sludging of blood through vessels. This produces pain due to hypoxia affecting sensory nerve endings, namely, the pain fibers. This of course hasn't been demonstrated or proven to date, but hypotheses usually precede discoveries. Also, it should be kept in mind that aspirin is anti-platelet and also anti-serotonin and imipramine is anti-platelet, anti-serotonin, and anti-cholinergic. In some patients whom we have studied at the height of their attacks, and even between attacks, there has been platelet thrombocytosis and, in some instances, abnormalities of platelet morphology. Perhaps imipramine and chlordiazepoxide in combination, which often relieve "tension headaches," are able to inhibit the activation of neurotropic virus; and perhaps they also have a direct or indirect effect by this inhibitory action on platelets of preventing hyperaggregation and/or sludging.

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It is hoped that this hypothesis can be tested and proved or disproved, preferably on the basis of an international cooperative study involving the collaboration of neurologists and immunologists.

Potential Changes Associated with Eye Movements in the Thalamus, Visual Cortex and Reticular Formation*

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Introduction

The major function of eye movements is to promote vision. This is accomplished by holding or moving the eyes so that targets are fixed on the retina. Visual acuity is high only in the center of the retina. Consequently, unless the eyes are pointing directly at a target, it is poorly seen. Thus, eye movements and attention are closely linked and the center of attention can frequently be inferred from knowledge of where the eyes are pointing, i.e., where a subject is looking.

The close relationship between vision, eye movements, and attention suggests that information about eye movements is widely distributed in the nervous system (1, 2). There is considerable support for this view, some of it from our studies of gross potential changes associated with eye movement in the thalamus, calcarine cortex, and pontine reticular formation (3–6). These studies will be summarized in this report.

Experiments were done on juvenile rhesus monkeys (Macaca mulatta). Bipolar stainless steel electrodes were implanted under anesthesia for stimulating and recording using stereotaxic techniques. Electrodes were placed in the optic tract (OT), lateral geniculate body (LGB), calcarine cortex, mesencephalic reticular formation (MRF), and in the paramedian zone of the pontine reticular formation (PPRF). Electrodes were fixed to the skull with dental acrylic resin cement. Monkeys were tested over periods of several months to several years. During testing they were seated upright in a primate chair with head held and arms and legs restrained. Eye movements were recorded by electrooculography (EOG). Animals received amphetamine (0.5 mg/Kg) to maintain alertness. Experimental methods and techniques for inducing and recording eye movements have been described in detail (3–7). At the end of experiments monkeys were killed with an overdose of anesthesia, brains were fixed, and electrode locations were determined in histologic sections.

Potential Changes in the Lateral Geniculate Body: Figure 1A shows potential changes recorded from LGB in light and in darkness during spontaneous eye movements. LGB potential changes in light are composed of two parts, a

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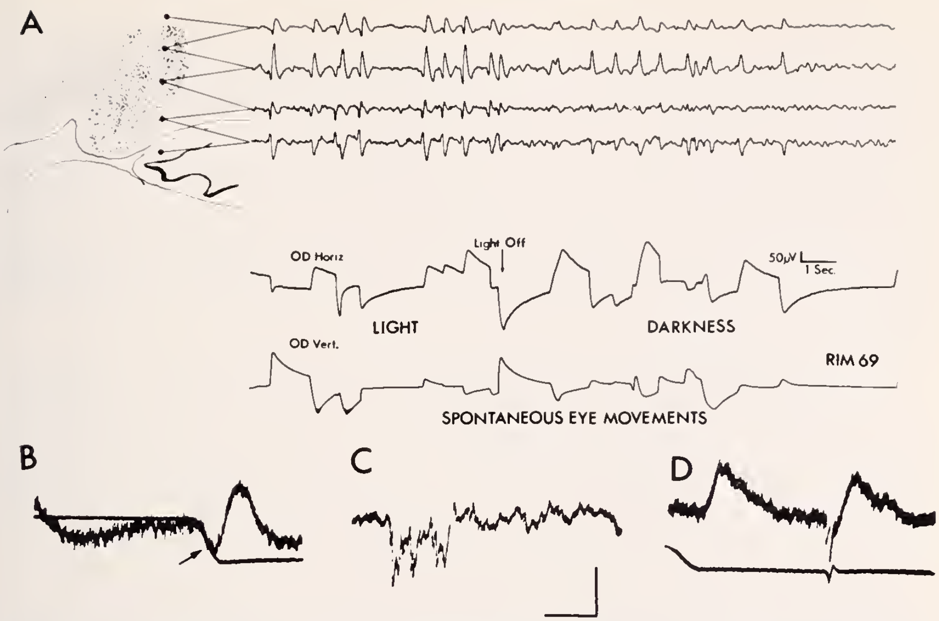

FIG. 1. A, Potentials occurring in LGB during spontaneous eye movements in light and darkness. On the right are bipolar recordings from points shown in the drawing on the left. Below are the EOG's showing horizontal and vertical movements in the right eye. The largest amplitude eye movements were about 45°. At the downward arrow (5th trace), the lights were extinguished and the animal was in total darkness for the remainder of the recording. Note the disappearance of the initial downgoing portion of the wave form when the animal was in darkness. Note also that the wave form of the LGB potential changes was not dependent on the amplitude or direction of eye movement. B, Biphasic LGB response during a saccade to the left in light. The thin trace is the EOG, thicker trace is monopolar recording from LGB. Upward arrow indicates initial downgoing positive portion of response which occurs in light. C, Positive LGB response to light flash on a faster time base. Eyes motionless. D, Negative LGB response during saccade to left in dark. EOG is lower trace. About 300 msec after the onset of the trace the brainstem was stimulated in the cross-hatched area shown in Fig. 6 (PPRF). A negative LGB potential was induced which was similar to that which occurred during the spontaneous saccade. Time base shown under C is 200 msec for B, 40 msec for C, and 100 msec for D. Vertical bar is 100 μV of amplitude for the LGB recordings of B & D, and 200 μV for C. It represents 30° of deviation for the EOG in B & D. (From Feldman & Cohen, J Neurophysiol 31:455–466, 1968.)

light-evoked response (Fig. 1A, light; 1B, arrow) followed by a light-independent potential change. The light-evoked response is positive and occurs about 30 msec after the eyes begin to move. It is not present in darkness. The light-evoked potential is of the same polarity as the response to light flash at these locations (Fig. 1C).

The light-independent potential is negative. It occurs about 35–40 msec after the onset of eye movement (Fig. 1A, darkness; 1D) and persists for approximately 120 msec. It also occurs if animals are paralyzed and given stimuli which would have induced eye movements (3). It is not evoked by pulling the eyes in the dark. Hence it is not due to activity originating in eye muscle proprioceptors. Rather it appears to reflect the activity of neural organizations which produced the eye movements themselves.

Eye movements can be separated into two general categories: slow movements and fast movements. Pursuit movements, tonic deviations, and slow phases of nystagmus are slow eye movements. Saccades and quick phases of nystagmus are rapid eye movements. LGB potentials are associated only with rapid eye movements and are similar whether the rapid eye movement is a quick phase of optokinetic or caloric nystagmus or a saccade. LGB potentials are also associated with blinks. LGB potentials do not accompany slow eye movements or periods of fixation. However, on occasion they can be evoked by auditory or somesthetic stimuli which are novel or startle the animal (3). Auditory and somesthetic projections to LGB have also been described in the cat (8).

Light-evoked responses to eye movement are widely distributed in LGB. In the dorsal small-cell layers the light-evoked potentials are smaller than the negative light-independent potential changes (Fig. 1A). Light-evoked potentials are more prominent in large cell layers of ventral LGB where there may be no light-independent potential changes (3). The amplitude and wave form of both the light-dependent and light-independent potential changes are not related to the direction or amplitude of movement (Fig. 1A).

Both types of potential changes associated with eye movement are found in other portions of the thalamus which are related to the visual system or to the reticular formation. Figures 2 and 3 show the results of mapping experiments

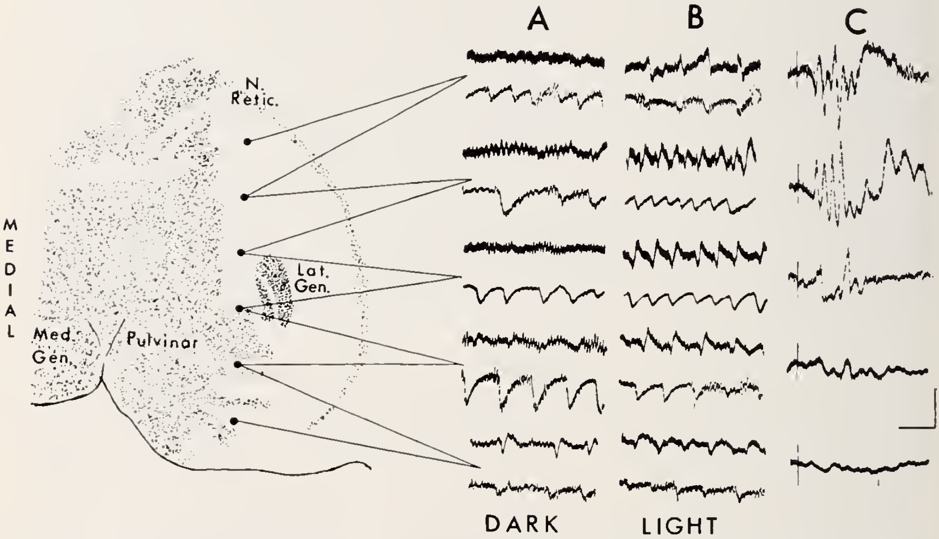

FIG. 2. Eye movement-associated potential changes in posterior thalamus, just medial to posterior portions of LGB. The top trace of each pair records the potential changes recorded bipolarly between points indicated on diagram and the bottom trace shows the horizontal EOG. The response to light flash is also shown. The time base is the horizontal bar which is 200 msec for recordings during caloric nystagmus and 40 msec for recordings during light flash. The vertical bar is 100 μV for the top trace and about 30° of horizontal deviation for the EOG. Note the absence of response in darkness at all recording locations except the bottom pair. Responses in light were prominent at each recording site in this electrode track. (From Feldman & Cohen, J Neurophysiol 31:455–466, 1968.)

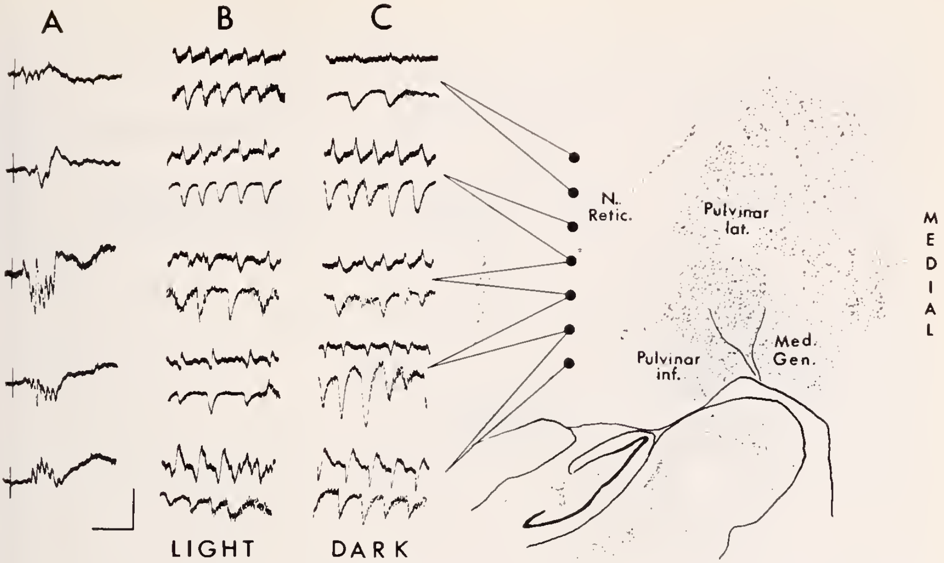

FIG. 3. Scheme similar to that of Fig. 2. Note the prominent potential changes in darkness at point in and close to Nucleus Reticularis. The potential changes in darkness reversed polarity between the 3rd and 4th set of recordings, suggesting that the source was located in this region.

done on alert, cervically transected monkeys who were free to move their eyes. Nystagmus was induced by caloric stimulation. A bipolar electrode was advanced through the thalamus in millimeter steps. The response to light flash and to eye movement in light and darkness was recorded at each electrode location.

In posterior portions of the thalamus between LGB and the pulvinar, a potential change was found to be associated with each rapid eye movement in light (Fig. 2B). This was predominantly a light-evoked response, since it disappeared when the animal moved its eyes in darkness (Fig. 2A). In lateral parts of the posterior thalamus, on the other hand, in the region of nucleus reticularis (Fig. 3) there was no difference in the potential changes associated with eye movements whether the animal was in light (Fig. 3B) or in darkness (Fig. 3C). The potential changes shown in Figure 3 more closely reflected efferent activity of the oculomotor system. In both thalamic regions, the potential changes were independent of the direction of eye movement and were similar regardless of whether the eyes had moved spontaneously during saccades or during induced nystagmus.

Potential Changes in the Calcarine Cortex: In the calcarine cortex of the monkey the major events associated with eye movement are light-evoked responses. An example of a potential change in the calcarine cortex induced by quick phases of nystagmus in light is shown in the middle trace of Fig. 4A. It was similar to potential changes induced at that site by light flash (Fig. 4B, middle trace). These potential changes did not occur when animals were in

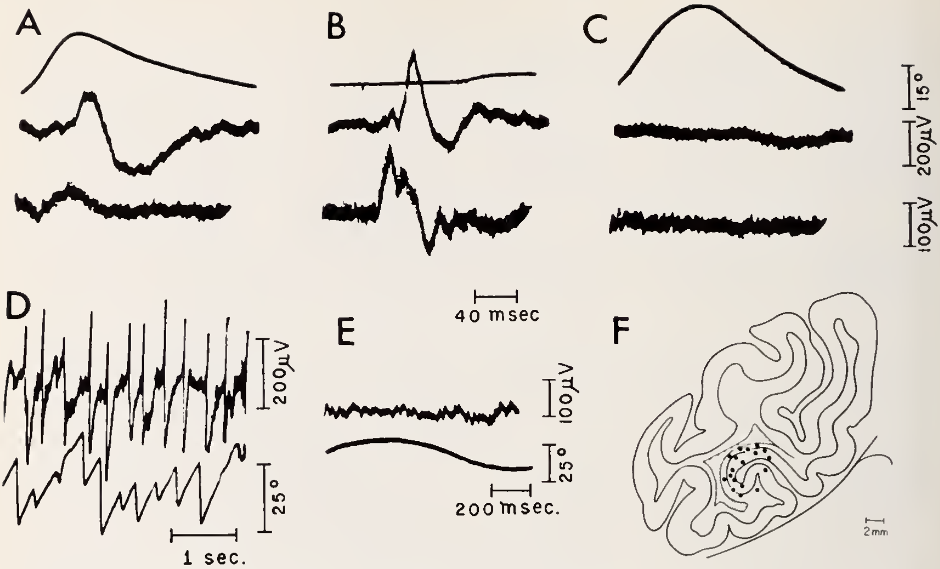

FIG. 4. Potential changes in the calcarine cortex and optic tract associated with eye movements. The top trace in A–C shows the horizontal EOG, an upward deflection indicating an eye movement to the right. The middle trace is a bipolar recording from calcarine cortex, and the bottom trace is a bipolar recording from the optic tract. A, Potential changes in optic tract and calcarine cortex associated with a quick phase of caloric nystagmus to the right in light. B, Potential changes associated with light flash recorded at the same locations. C, Quick phase of caloric nystagmus in darkness. No potential changes were induced in the optic tract and calcarine cortex. D, Calcarine potential changes associated with quick phases of optokinetic nystagmus. E, No potential changes were induced in the calcarine cortex (upper trace) during pursuit movement (lower trace). F, Locations of recording electrodes in the region of the calcarine cortex in 15 monkeys. Each dot shows the position of the tip electrode. Section is in the A-15 vertical stereotaxic plane. This portion of the calcarine cortex receives projections from the periphery of the retina. The cross-hatched area shows the region of fibers reach the calcarine cortex. (From Cohen & Feldman, Exp Neurol 31:100–113, 1971.)

darkness (Fig. 4C). The bottom traces in Fig. 4A–C show concurrent recordings from the optic tract. Potential changes in OT associated with eye movement are also light-evoked responses (Fig. 4A) and are not present in darkness (Fig. 4C).

Potential changes in the calcarine cortex are similar whether induced by saccades or quick phases of OKN or caloric nystagmus, but are not induced by pursuit movements (Fig. 4E). These light-evoked potential changes in the calcarine cortex are very prominent during nystagmus (Fig. 4D). They have very little dependence on contrast in the external visual fields (6). Electrode locations from which calcarine responses were recorded are shown in Figure 4F. These portions of the striate cortex receive afferent information from the periphery of the retina (9). Coded into the latency of these potential changes is the absolute level of illumination, while the amplitude reflects the amount of light which reaches the retina and the size and velocity of the rapid eye movements which induced these potential changes (6).

Potential changes (lambda waves) and unit activity (10) associated with eye movement are present over lateral portions of the occipital lobes which receive projections from the fovea (9). Characteristics of lambda waves and a comparison of lambda waves with calcarine potential changes associated with eye movement are reviewed in Reference 6. Contrary to calcarine potential changes, lambda waves are dependent on strong contrast and on interest of the subject in the external visual field. Unit activity over the lateral cortex also disappears if external contrast is reduced, even though the eyes move in light (10).

Potential changes which reflect efferent activity of the oculomotor system in the visual cortex are prominent in the cat (11–13), but also occur in the monkey. An example is shown in Fig. 5. The top trace is a bipolar recording from electrodes located in the calcarine cortex in the cross hatched area of Fig. 4F. The second trace is a bipolar recording from electrodes in LGB, and the third trace is the horizontal EOG. Similar potential changes were produced at these recording sites in LGB and calcarine cortex by saccades whether the monkey was in light (Fig. 5A) or in darkness (Fig. 5B). They were opposite in polarity to responses to light flash recorded at these same locations (Fig. 5C). LGB and calcarine potential changes associated with quick phases of caloric nystagmus with the monkey in light are shown in Figure 5D. The monkey was paralyzed with succinylcholine and artificially respired. LGB and calcarine potential changes, similar to those recorded in darkness (Fig. 5B) were induced by caloric stimulation with the monkey in light (Fig. 5E) and in darkness (Fig. 5F). This shows that the potential changes recorded at these locations were

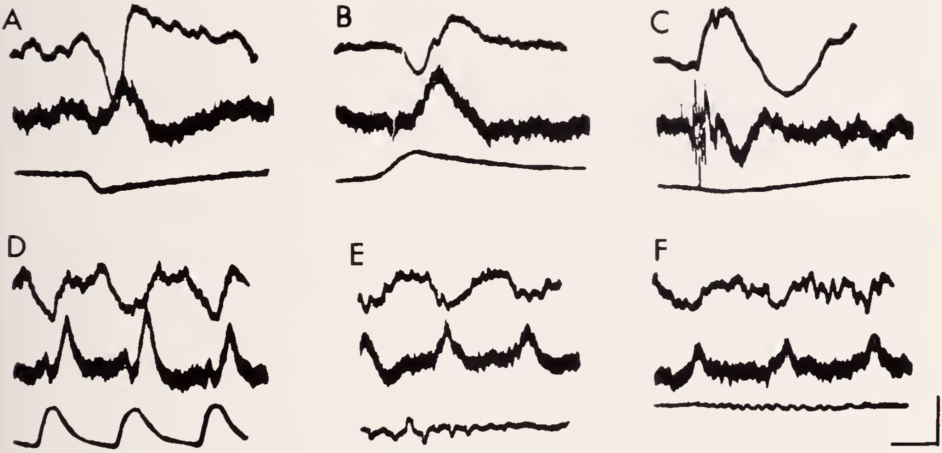

FIG. 5. Calcarine cortex (upper traces) and LGB potential changes (middle traces) associated with rapid eye movement. Horizontal EOG is the bottom trace. A, Calcarine cortex and LGB potential changes in light, and B, in darkness. C, Potential changes evoked at these locations by light flash. D, Potential changes in calcarine cortex associated with quick phases of caloric nystagmus in light. After the animal was paralyzed caloric stimulation again induced repetitive wave forms in the calcarine cortex in light (E) and in darkness (F). The EOG was increased 10 times in E and F to demonstrate the absence of eye movement. The vertical bar is 30° for the EOG and 200 μV for the LGB and calcarine recordings. The horizontal bar is 100 msec for A–C and 200 msec for D–F.

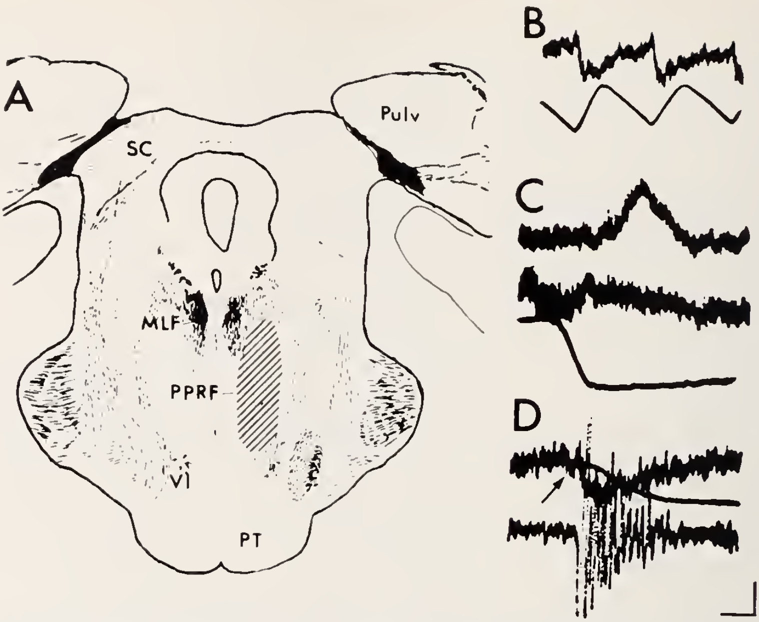

FIG. 6. A, Diagram of brainstem showing location of PPRF on one side (cross-hatched area). This section is through the midpons in the AP — 0.5 vertical stereotaxic plane. SC, Superior colliculus; MLF, Median longitudinal fasciculus; Pulv, Pulvinar; PPRF, Paramedian zone of the pontine reticular formation; VI, 6th nerve rootlets; PT, Pyramidal tract. B, Potential changes in left PPRF (top trace) during quick phases of nystagmus to right (bottom trace). C, Recordings from LGB, top trace; PPRF, middle trace; EOG, bottom trace during quick phase of caloric nystagmus to left. The potential change in PPRF preceded the quick phase of nystagmus anad the LGB potential change. D, Potential change in left PPRF (top trace) during saccadic eye movement to left (middle trace). EMG of left lateral rectus muscle is shown in bottom trace. The time base shown by the horizontal bar under D is 200 msec for B, 40 msec for C, and 20 msec for D. The vertical bar is 200 μV for the LGB recording and 100 μV for the PPRF recording. Vertical bar also shows about 30° of deviation for the horizontal EOG. (From Cohen & Feldman, J Neurophysiol 31:806–817, 1968.)

neither light-dependent nor were they dependent on afferent activity arising in eye muscle proprioceptors.

Potential Changes in the Paramedian Zone of the Pontine Reticular Formation (PPRF): Oculomotor function has been found by Bender and coworkers to be strongly represented in portions of the pontine tegmentum which lie just lateral to the midline (cross-hatched area of Fig. 6A) (14–16). Small lesions of this region produce conjugate paralysis of gaze to the ipsilateral side (14–18). Stimulation of this region induces strong ipsilateral horizontal eye movements at short latencies (16, 19). Potential changes (4) and unit activity (20, 21) associated with every rapid eye movement and blink in light or in darkness are also found in the PPRF (Fig. 6B). Since the PPRF potential changes and unit activity lead each rapid eye movement, they could not be light-evoked responses nor could they be dependent on activity arising in eye muscle proprioceptors. Rather it is likely that saccades and quick phases in the horizontal plane are generated in the PPRF (18, 19, 21).

The comparative latencies of PPRF and LGB potential changes are shown in Fig. 6C. The PPRF potential leads the eye movement, the LGB potential change follows it. The sequence of activity is shown in Fig. 6D. First there was activity in the PPRF (top trace, arrow), then the left lateral rectus muscle was activated (bottom trace), then the eye moved to the left (middle trace).

If the PPRF is electrically stimulated, potential changes are produced in LGB (Fig. 1D, end of trace) which are similar to those which occur naturally (Fig. 1D, beginning of trace) (4, 5, 22). This suggests that activity responsible for producing the LGB potential changes arises in the PPRF (4). The ascending pathway for this activity is probably through the MRF since similar LGB potentials can also be induced by MRF stimulation (4, 5).

Comment

These studies show that there are at least two types of potential changes associated with eye movements in the visual system. One reflects the activity of the central neural organizations which produce rapid eye movements and blinks. The second is a light-evoked response generated in the retina by the movement of the eyes. A third type of potential change is induced by eye muscle proprioceptors in the cerebellum as a result of stretching of the eye muscles (23).

The functional significance of eye movement associated potentials is not clear. In the pons the recorded potential changes probably reflect activity of neurons which generate the eye movements and activity of neurons responsible for producing the light-independent potentials in other parts of the brain. Thalamic and cortical potentials, on the other hand, may reflect mechanisms responsible for saccadic suppression (24) or for maintenance of the stability of the visual world (1, 2, 25). Brainstem activation of LGB could be mediated via the pre-geniculate nucleus since it receives projections from the brainstem, and cells in this nucleus fire in association with eye movements (26) at about the same latency as the light-independent LGB potential changes (3).

There are at least two separate mechanisms by which changes in visual threshold could be affected during saccades. One is retinal in origin. Movement of images at certain velocities over the retina causes inhibition of cells in the visual pathway (27) and an increase in the threshold of light perception (28). Secondly, there is activation of LGB neurons during eye movements (29, 30). Many of these are probably inhibitory interneurons in LGB (30) which are known to cause postsynaptic inhibition of geniculo-calcarine relay cells (31). However, changes in the threshold of light perception during saccadic suppression are relatively small, and do not appear to account for the lack of perception during eye movements. Moreover, there is little or no change in transmission through LGB during rapid eye movements (5, 32) in the alert monkey. Therefore, other central mechanisms must be sought to explain the imperception which occurs during rapid eye movements and blinks.

A second possible function for the potential responses in the visual system is that they are part of a mechanism for stabilizing the visual world. That is, they may reflect activity originating in the reticular formation which signals to the visual system that a rapid eye movement has occurred, and therefore that there has been a shift in the position of the retina and in the incoming visual information (1, 2, 25). In this regard it is of interest that blinks cause a similar type of potential change throughout the visual system, while slow eye movements, i.e., pursuit movements and slow phases of nystagmus, do not induce these potential changes. Presumably the continuous monitoring of incoming visual information during slow eye movements precludes the necessity for this type of response.

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A Physiological Study of Rostral Dominance in Simultaneously Applied Ipsilateral Somatosensory Stimuli

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Double simultaneous special and somatosensory testing was introduced into modern neurology as an important heuristic indicator of brain dysfunction by Bender (1). By the study of lateral dominance in unilateral brain injuries he demonstrated the effect of perceptual rivalry (2). Later the study of responses to simultaneously applied ipsilateral somatosensory stimuli became a prime index of sentiency, or vigilance, in individuals with unilateral and/or diffuse brain dysfunction (3–6).

This presentation will focus attention on the response effect of simultaneous ipsilateral stimulation of two widely separated body surfaces. It was early observed in the presence of brain dysfunction that when two or more stimuli were applied ipsilaterally to disparate body surfaces there was a fairly orderly failure to perceive more than one stimulus. Even normal individuals under novel conditions show this phenomenon. The failure to resolve simultaneously applied stimuli formed the basis of the face-hand test of Bender (3). In patients with focal or general brain dysfunction, the failure to perceive occurred predominantly in the more distally (from the head) applied stimulus, i.e., in the face-hand combination only the face stimulus was generally recognized; in the shoulder-hand combination only the shoulder stimulus was usually reported, and in the hip-ankle combination only the hip stimulus was perceived. On these bases, Cohn (4) designated this phenomenon as rostral dominance. When stimuli were applied simultaneously in the hand-knee combination some investigators found that the knee dominated the hand (3). In my own series of patients it appeared that statistically there was no remarkable predominance of the knee over the hand when stimulated simultaneously, but there was a tendency for the hand to dominate the knee in a ratio of around 3:2.

It was thus deemed of some importance to physiologically investigate some factors that might be explanatory for the observation of rostral dominance. This was accomplished by stimulating two peripheral nerves simultaneously and recording the resultant summated evoked EEG which was derived synchronously from each cerebral hemisphere.

Method

The ipsilateral median and tibial nerves were percutaneously stimulated separately, and then simultaneously by 0.1 msec rectangular pulses. The stimulation current was adjusted to

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achieve slightly more than just perceptible movement of the appropriate muscles; during the testing the currents were adjusted if, and when, necessary to maintain uniform responses. The summated cortical evoked EEG was recorded from each cerebral hemisphere by electrodes placed in the temporal areas 3 cm in front of the upper insertion of the ears; the other electrode on each side was in the mid-coronal plane 4 cm lateral to the mid-sagittal line. These electrodes effectively covered the surface projection of each sensory cortex. The amplifiers were BME 111A which were operated essentially flat between 1 and 2500 hz, and with an overall time constant of the system of 0.4 sec; only one capacitor was used in each amplifier system. No phase shifts of the amplifiers were detectable in the frequency range employed. The system gains were amplitude matched prior to each testing. The amplified cortical outputs were summed by means of a CAT 400A operating with 250 msec analysis times; and with variable full scale sensitivities. Calibrations were made by passing a single train of sinusoidal waves of known voltage into the CAT under the fixed conditions of operation. No attempt was made to study recovery cycles as the experimental design was to replicate the conditions of neurological testing.

Results

When ipsilateral median and tibial nerves were simultaneously stimulated in 30 subjects with normal central and peripheral nervous system function, it was observed that 3 in each 4 individuals had evoked EEG patterns whose contours were basically similar to the wave-forms seen when the median nerve alone was stimulated. The remainder of the subjects showed a major pattern that resembled the control contours when only the tibial nerve was stimulated. In nearly all cases however (single and double stimulation) the major peaks of the summed evoked EEG derived from the side contralateral to the peripheral stimuli showed the median nerve evoked cortical pattern to have a latency ranging between 12 to 35 msec shorter than the major peaks evoked by tibial nerve stimulation. But as noted above, about one-fourth of the subjects had such relatively high voltage-evoked EEG activity from the tibial nerve stimulation that despite the earlier appearance of the median nerve evoked peaks, the pattern of the isolated tibial nerve stimulation response predominated. These findings are illustrated in the following figures.

Figure 1 shows that with right median nerve stimulation the first positive peak over the contralateral left brain (lower trace of the first pair of lines) occurred nearly 35 msec prior to the major positive peak evoked by tibial nerve stimulation (lower trace in second pair of lines). It is observed that the summated cortical-evoked EEG pattern resulting from stimulation of both nerves had the basic contour seen when the median nerve alone was stimulated (lower trace in the third pair of lines).

In figure 2 stimulation of the right median nerve again resulted in a contralateral summated evoked EEG which had the major initial positive peak at least 15 msec prior to that observed with tibial nerve stimulation. Again the amplitude of the evoked EEG was greater from median nerve stimulation than from the tibial nerve stimulation. The cortical evoked EEG resulting from the simultaneous stimulation of these peripheral nerves showed major contours similar to the cortical response when only the median nerve was stimulated.

Figure 3 shows the overall similarity of evoked EEG patterns in successive testings. Although there was some variability of the summated cortical evoked EEG there was no doubt that the primary patterns were reproductive. In each instance the latency of the median nerve initial peaks were approximately 12 msec shorter than from tibial nerve stimulation. As before, the patterns of the

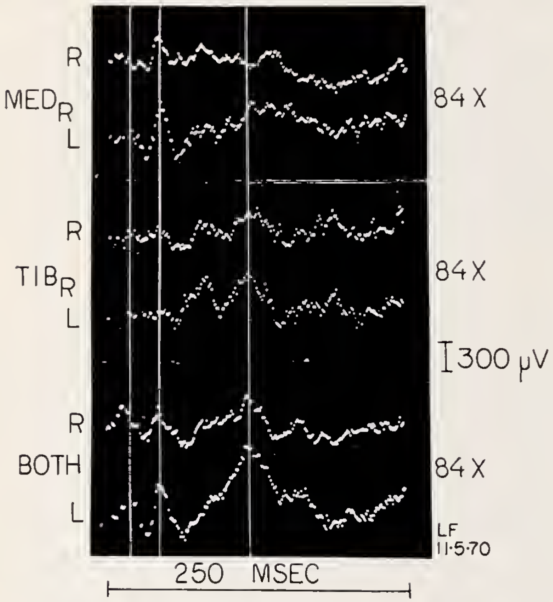

FIG. 1. Right median nerve stimulation (upper two lines). Synchronous right and left summated cortical evoked EEG. Second pair of lines, right tibial nerve stimulation response. Lowest pair of lines, evoked EEG resulting from simultaneous stimulation of right median and tibial nerves. 84× represents the number of stimuli summed. Analysis time 250 msec. Calibration 300 microvolts. Positive deflection is up. Simultaneous ordinates erected from major peaks of the median nerve stimulation response.

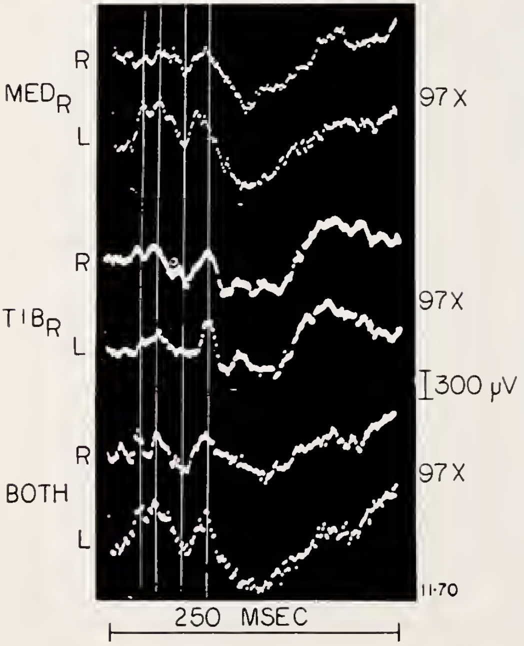

FIG. 2. Evoked EEG resulting from right median nerve stimulation (upper two lines). Middle two lines, bilateral evoked EEG from right tibial nerve stimulation. Lowest two lines, evoked EEG to simultaneous median and tibial nerve stimulation. Analysis time 250 msec; 97 stimuli for each summation. 300 microvolt calibration.

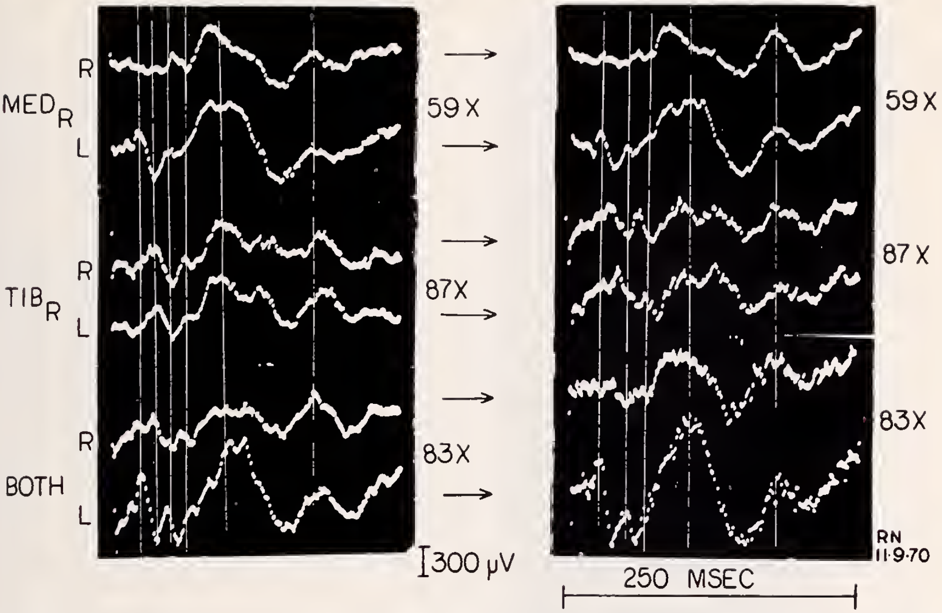

Fig. 3. Consistency of evoked EEG patterns. Upper two lines on each side of figure represent responses from right median nerve stimulation. Middle two lines on each side shows evoked EEG to right tibial nerve stimulation. Lowest sets of lines are evoked EEGs to simultaneous stimulation of both nerves. Number of summed stimuli denoted by numerals to the right of each pair of lines. Analysis time 250 msec; calibration 300 microvolts.

summated evoked EEG resulting from simultaneous stimulation of both nerves had the basic character of the median nerve responses.

In Figure 4 the relatively low amplitude peaks of the summated evoked EEG to contralateral median nerve stimulation occurred approximately 20 msec prior to the first major peak resulting from tibial nerve stimulation. Despite the difference in the time of peaking, when both contralateral nerves were stimulated the resulting summated evoked EEG had an overall pattern almost congruent with that observed when only the tibial nerve was stimulated. The stability of the evoked EEG which resulted from simultaneously stimulating both nerves is shown in the successive recordings of the figure (third and fourth pairs of waves) designated as BOTH.

Discussion

Of course the repeated stimulations necessary in this model were not precisely similar to the conditions of clinical testing. The neurologic test generated an immediate, isolated response to simultaneously applied tactile or pinprick stimuli over ipsilateral body surfaces. The assumption made in this presentation is that the summated evoked, and the immediate clinical responses activate the same cortical elements; and that the cortical-evoked EEG's are only of decreased amplitude, with the same time course as the immediate response which generated the observed clinical phenomena. From these studies of the

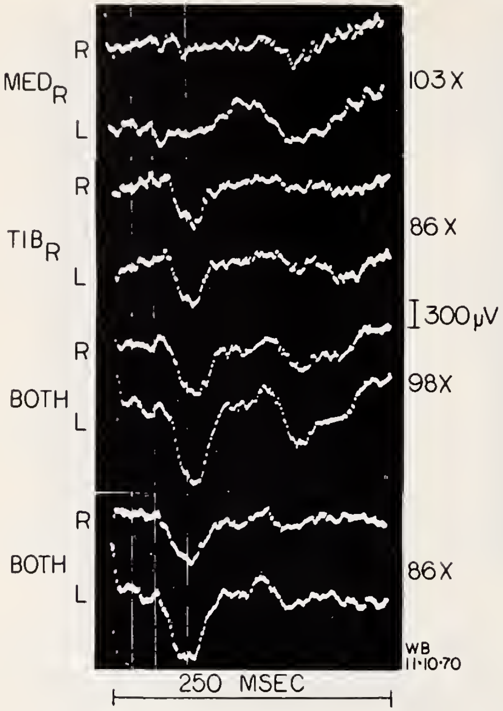

Fig. 4. Evoked EEGs to right median nerve stimulation (upper two lines). Evoked EEGs to right tibial nerve stimuli (next two lines). Evoked EEGs to simultaneous right median and tibial nerve stimulation (lowest two pairs of lines). Number of summed stimuli indicated by numerals. 250 msec sweep. Calibration 300 microvolts.

summated evoked EEG it seemed that there was nearly always a differential delay in the impulse arrival times from the disparate areas of stimulation. But it appeared that the actual delay times of peaks resulting from the various areas stimulated were not the primary determinants of clinical response, but that the amplitude of the evoked EEG's, which had individual variations, seemed to be a more definitive element for recognition since the amplitudes of summed cortical output correlated best with the neurological data.

In this work the experimental design was not to specifically demonstrate a direct clinical and electrical correspondence of rostral dominance. The implication, however, was that if specific testing was employed such direct correspondence might be recognized. It thus appears that in normal individuals the time course, and particularly the amplitude characteristics of the cortical response, determine the reception and consequent recognition of the paired stimuli. In patients in whom there is generally impaired discrimination function, this physiological bias toward the more rapid time of arrival and the greater amplitude of cortical response from contralateral peripheral nerve stimulation almost forces a rostral dominance. A similar investigation of the face-hand combination will be made.

Conclusions

With simultaneous median and tibial nerve stimulation three-fourths of the normal subjects studied had evoked EEG's with patterns similar to those obtained with stimulation of the median nerve alone. One-fourth showed evoked EEG patterns from simultaneous peripheral nerve (median and tibial) stimulation that were similar to the pattern when the tibial nerve alone was stimulated. The evoked EEG responses indicated that a major determinant of the perception, and consequent resolution of simultaneously applied stimuli was the amplitude of the evoked cortical potentials.

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Misoplegia, or Hatred of Hemiplegia

MACDONALD CRITCHLEY, M.D.*

Misoplegia is a term applied to the phenomenon whereby a hemiplegic patient develops a morbid dislike directed towards the offending immobile limbs. It is not a hostile or paranoid reaction towards the actual fact of his being crippled or paralyzed. It must be granted that misoplegia is not a common state of affairs. Perhaps it is more frequently found in patients with a left hemiplegia than with a right, assuming, of course, that they were right-handed before they sustained their brain lesions.

Since Babinski's original description in 1914 (1), we have been familiar with the conception of an anosognosia whereby a left hemiplegic, after an abrupt paralysis, is obviously temporarily unaware of the fact that his limbs are paralyzed. This, of course, is a commonplace reaction, but only when the cerebral lesion is of an acute or fulminating character. If the patient's sensorium is cloudy, he may go further and proceed to deny the fact that he is paralyzed when directly questioned. As yet, neurologic nomenclature holds no single term to stand for this denial of hemiplegia. Superimposed upon this phenomenon, and from searching cross-examination at the bedside, it may be found that the patient is developing various degrees of confabulation. That is to say, when directed to move his affected limb, he may proclaim he has done so, when indeed he has done nothing of the sort. Or he may move the opposite limb and assume or attempt to persuade the examiner that he has satisfactorily flexed or extended the paralyzed limb. Or, in a most bizarre fashion, he may deny actual ownership of the limb. As the interrogation continues, and he is asked to whom the paralyzed limbs belong, he may resort to confabulatory projection and proclaim that the paralyzed arm and leg are the limbs of someone else, close by or remote, real or imaginary.

This anosognosia, so well described and elaborated by the studies of the Mount Sinai School of Neurology, tends to fade in the ensuing days or weeks after the acute onset of the illness. The change in attitude is, in part, due to the intervention of nurses in attendance, visiting relatives, and the doctor in charge of the case. In other words, the patient eventually becomes aware, not only that he has a left arm and leg, but that it is actually helpless and out of control.

Here again, we may view at the bedside a number of diverse types of confabulation, implicated with various anomalies of corporeal awareness or body-image. Thus, the patient may perhaps admit grudgingly that he is well and truly disabled. However, he may seek to explain away the disability onto some peripheral disorder, fancied or factual, but in the latter case quite insignificant and irrelevant. Thus the patient may attribute his paresis to an arthritis, a sprain, or an old fracture. This again is a transient state of affairs. Later he may come to accept the paralysis for what it is, a hemiparesis of cerebral origin. However, he displays not so much a euphoric attitude as a light-hearted acceptance of the disablement, shutting his eyes and ears to the probable repercussions of his stroke regarding his future employability and wage-earning capacity. In other words he treats his illness in a peculiarly carefree, even jaunty fashion, and may even joke about the curious heaviness, or awkwardness of the affected arm and leg. Again, this phenomenon was also described by Babinski, under the term "anosodiaphoria," which literally means "lack of concern over the existence of a disability." This frame of mind may persist and may prove detrimental to the success of his treatment in that the patient may fallaciously regard reeducation and rehabilitation as quite unnecessary.

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* Dr. Critchley has shared Dr. Bender's interest in problems of language and perception for many years.

An even more common and very intriguing experience is that which I have described as "personification of the paralyzed limbs." Developing sometime after an initial anosognosia, it can in some ways be regarded as a diametrically opposite attitude, perhaps a pattern of overcompensation. A patient so affected has now come to accept the paralysis as a fact, but he appears to be almost detached about it, something of an onlooker. The paralyzed arm and leg come now to be regarded almost as something apart from the rest of the anatomy, as if it were a foreign body. The useless limbs are referred to as though they were invested with a personality or identity of their own, but in a semi-facetious and proprietary fashion, as one might perhaps show towards a plaything or pet. The patient may speak of his arm and leg in a sort of baby-talk. A hemiparetic male may refer to the paralyzed arm as "she" or "it," while a female patient more often speaks in terms of "he" or "him". More often still nicknames are attached to the affected limbs in a childish fashion. I have personal knowledge of patients who have called their paralyzed arm "George," "Toby," "silly Billy," "lucky Joe," "Baby," "Gammy," "Hermione," "the immovable one," "the curse," "lazybones," "the nuisance," "old useless," "silly Jimmy," "floppy Joe," "Fanny Ann," and "Dolly Gray." A patient recorded by Gilliat and Pratt referred to her paralyzed arms as a "poor little withered hand" and would often pick it up and kiss it. An Australian designated his limb as "the bugger"—a term of affection in the Antipodes. Another patient dubbed his paralyzed arm as "the Communist," by which he meant the limb that refused to work. To another patient the paretic left arm was "James," and the leg "lefty."

I suspect that this phenomenon of personification is far more common that we neurologists realize, and family doctors, physiotherapists, and the relatives are perhaps better informed. It is odd that until my original description in 1955 (2) it had not been referred to in the literature of neurology.

Personification of this kind often coexists with some degree of anosodiaphoria or abnormal cheerfulness over the existence of a hemiplegia. This is shown by the nature of some of the foregoing nicknames. I think it likely that in every case unawareness of the hemiplegia comes first, perhaps even a temporary denial of the paralysis. As in the case of anosognosia, personification is commoner in left hemiplegics than in right hemiplegics. Nonetheless, I have encountered at least one such case in a right-handed man who had developed a mild dysphasia and a right-sided paresis, the lesion presumably being in the dominant cerebral hemisphere.

Sometimes the personification becomes part of a frank delusion. A man with a right temporo-parietal meningioma, speaking of his weak left upper limb, said "He gets tired sometimes; he doesn't keep time with you; he gets out of step. He gets very lazy; he sits and hangs about and when he does get hold of you he doesn't want to leave you. He's been doing this for a week." Asked to open his fist, he held it up before him, still clenched, and then began to cuddle and caress it, patting it and rubbing it, talking to it and encouraging it—"Come on, you little monkey, don't let us down. Come on, 'Monkey.' I used to call him 'Lucky.' we're doing nicely now, so we'll call him 'Lucky.' Come on, 'Lucky.'" After nursing and cajoling the left hand and arm for about three minutes, the fingers began to extend. Asked what was wrong with the left hand, the patient explained, "he doesn't know what the other one's doing; he's so slow, sitting idle". The nursing staff observed that at meal-times he would "feed" the "little monkey" with a spoon, saying, "come on, have a bit."

Apart from hemiplegia, patients with nonneurologic disease rarely adopt this curiously detached attitude towards painful or crippled extremities. I can recall one or two exceptions, however. Thus, when the librettist W.S. Gilbert was unwell, he was in the habit of addressing his gouty limbs as "Labouchere" and "Clement Scott," who were the respective editors of "Truth" and "The Theatre."

Amputees very occasionally personify their phantom limbs. Perhaps the most bizarre, nonneurologic example which came my way was a patient who spoke of his colostomy as "George."

In observing the diverse instances of personification shown by hemiplegics when they are thinking or talking about their affected arms, it is obvious that many patients adopt anything but an affectionate or even wholly detached attitude. Some patients assume a frame of mind towards their paralyzed segments which is critical, disapproving, or hostile. This is mirrored in such terms as "the delinquent". One hemiplegic patient designated with bitterness her affected arm as "the dummy," and she characterized herself as "a broken doll." A doctor patient of mine was a keen musician with strong likes and dislikes. When he developed a left hemiplegia, he specified his paralyzed left arm as "Schumann," explaining that "he was not much good," whereas the normal right arm was "Chopin," for whom he had a profound admiration. When, as a visiting professor to Melbourne, I was examining a hemiplegic female patient, she suddenly proclaimed in the words of St. Paul, "Oh! wretched woman that I am! Who will deliver me from the body of this death?".

These critical attitudes are the minor manifestations of what I have called a misoplegia. From this mildly paranoid reaction, frankly psychotic types of thinking and behavior may insidiously develop. In a patient whose left hemiparesis was mild, but was complicated by a gross loss of postural sensibility and a hemianopia, the affected arm would spontaneously wander around in space—often without the victim's knowledge. The patient was heard to address his affected limb in the following terms: "You bloody bastard! It's a lost soul, this bloody thing. It keeps following me around. It gets in my way when I read. I find my hand up by my face, waving about."

A female patient who sometimes denied the ownership of her paralyzed arm, while at other times reluctantly admitting that it was hers, would often thrust it violently away from her, out of her sight.

Still more hostile was the persecutory type of personification shown by a patient whose paretic left arm was frequently the seat of focal involuntary jerking movements. The patient confided to the doctor, "The old swine! The stinker! Don't talk about it, it might hear you and start twitching again."

Not infrequently, the patient develops illusions as to the size and appearance of the affected limb, often describing it in extravagant language. "Like a bird's claw"; "Like a heavy iron bar"; "A piece of dead meat"; "Wooden . . . it won't cooperate"; "Shriveled and shrunken, like the hand of a mummy." Sometimes the patient will endeavor to conceal the limb under a shawl, or the jacket of a coat, or the bedclothes. At times, he will avert his gaze by turning his eyes, head and neck towards the opposite direction. Not infrequently, the patient may be observed to violently strike the affected hand, over and over again, screaming abuse at it. This is misoplegia in the highest degree.

What underlies these variable attitudes on the part of the patient towards his disablement, which may run through the gamut of unawareness, denial, unconcern, disownment of the limb, somatoparaphrenia, insouciant acceptance, affectionate personification, paranoid hatred, and delusional ideas as to the physical properties of the limb? No dogmatic answer can be made, but many considerations arise which should be studied in depth.

In discussing this problem, one might well refer first of all to the more serious question of the varying modes of reaction of a patient rendered completely blind from an abrupt cerebral lesion. Reaction to the disability, we find by experience, varies from simple unawareness of the visual defect, to blatant denial of the blindness, confabulation, reluctant admission that the sight has perhaps deteriorated to a minor degree, or projection of the disability to the environment such as by claiming inadequate illumination. We also see in the sphere of defective sight from cerebral insult something which we might call "visual anosodiaphoria."

These reactions follow the same pattern as the hemiplegic, but these unexpected reactions are more striking in the blind than in the crippled. There are several hypotheses which have been put forward in an attempt to explain some of these various attitudes towards the disability of blindness or paralysis.

In the first place there is the notion that anosognosia, whether for blindness or hemiplegia, is a *specific manifestation of involvement of the parietal lobe,* particularly on the nondominant half of the brain. In other words, it is quintessentially a focal manifestation.

On the other hand, we have the observations of Weinstein and Kahn (3), who reject the idea of anosognosia as an expression of a focal parietal lobe lesion.

While parietal lobe damage produces a lateralized somatosensory deficit, they find that enduring denial and the other alterations of behavior that invariably occur in anosognosia involve a more extensive disturbance of brain function. Denial of illness may occur with normal brain function, and the brain dysfunction determines the *pattern* in which it is expressed. Weinstein and Kahn also stressed the importance of the premorbid personality in determining the content of denial. They state that to a considerable degree denial is the product of a dyadic interaction between patient and examiner. A patient may deny his hemiplegia in one context of language and not another and may deny his illness to a doctor but admit it to a relative. Jaffe and Slote (4) interviewed some of Weinstein and Kahn's patients and found that the degree of the denial was influenced by the examiner's attitude. They also concluded that "anosognosia represents a symbolic adaptive mechanism to be observed under the conditions of communicative interreaction."

The idea that the lack or presence of insight into the effects of a cerebral lesion depend almost entirely on the premorbid personality is found in a phrase within the Koran, "your illness is within you but you don't see it." A century ago, Hughlings Jackson repeatedly referred to this possibility. Speaking of the "four factors of the insanities," Jackson had in mind the clinical consequences of a localized lesion of the brain, and not necessarily a psychotic state. According to his teaching, the effects depended on: 1) the site of the lesion; 2) the size of the lesion; 3) the degree of abruptness of onset; and 4) the kind of brain that existed prior to the appearance of the lesion. It is this fourth factor that may be argued as being all-important in determining the nature of the clinical picture.

In misoplegia, we may find the hypothesis of the Mount Sinai School the most acceptable one, although other explanations are not necessarily excluded. Morbid dislike of the paralyzed extremities may prove to be evidence of a hypochondriacal notion bordering on the delusional, which is, in turn, the operation of an acquired cerebral lesion upon a susceptible premorbid personality. Such an individual has perhaps always been an obsessional and a candidate for hypochondriacal trends by reason of an innate preoccupation with bodily efficiency and physical fitness. Perhaps the patient was once a man of exceptional muscular prowess and of athletic proclivity. We know that by easy stages this may in later life lead on to a certain faddiness, eccentricity, or crankiness. The sudden development of a hemiplegia in such a subject will then constitute much more of an affront to the *amour-propre*, one which would scarcely occur in the case of the "cracked pitcher," the weakling of indifferent physique. The novelist, Christopher Morley, was shrewdly correct in the statement which he put into the lips of Kitty Foyle: "When anything goes wrong with a man, he sure lets you hear about it. If they'd been athletes, I guess they just think of bodies as something to have fun with until the works begin to gum up. They don't realize the way women have to that it's a damn complicated piece of doings."

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Observations on the Frog Dorsal Column Nucleus

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Introduction

Even though the frog is so commonly used for electrophysiologic and pharmacologic investigations, the literature contains surprisingly few references to the amphibian sensory system. In part this is due to conflicting statements about whether or not distinct lemniscal sensory pathways exist in amphibians (1, 2). However, evidence is available which suggests that there is an organized dorsal column system ending in the medulla (3–6). This study was undertaken to gain information about this sensory system using electrophysiologic methods; and the results suggest that, even in the simple primitive amphibian medulla, primary afferent depolarization is present to modify the transmission of information through the first synaptic sensory relay.

Methods

Most experiments were carried out on adult Rana pipiens, 6–8 cm in body length, lightly anesthetized by immersion in tricane methanesulfonate (MS-222, Ayerst Laboratories, 1 g/l). After mounting the animals in a modified stereotaxic apparatus by means of jaw clamps and after inserting pins into the spinal column and sacrum, the thalamus, brain stem, and cervical spinal cord were exposed by an extensive craniotomy and laminectomy. The dura was slit and the exposed neural tissue protected with mineral oil. The skin was kept moist. Several cutaneous and mixed fore- and hind-limb nerves were cut distally for bipolar stimulation or recording. Glass capillary micropipettes (4M NaCl) were used to antidromically stimulate the central endings of primary afferent fibers. Medullary surface potentials were recorded using Teflon®-coated tungsten electrodes (25 or 100 μ). Conventional differential recording and display techniques were used. Experiments were carried out at room temperature (21–23°C).

Results

Surface Recording: Histologic investigations of the frog medulla have demonstrated a small but well-differentiated nuclear collection of neurons which we will refer to as the dorsal column nucleus (DCN, unpublished observations). This nucleus is located between the obex and the second spinal root† and within a mm of either side of the midline. Stimulation of a cutaneous or a mixed nerve evoked a complex potential which could be recorded when a monopolar electrode was placed on the dorsal surface of the medulla overlying the DCN (Fig. 1A). This DCN surface potential consisted of three sequential waves: an initial diphasic spike, a following negativity, and a later positivity. All three waves showed an amplitude which was proportional to the intensity of the stimulating current and reached a maximum at 4-6x threshold. This compound potential resembles potentials recorded from the surface of the cat dorsal column nuclei (8, 9) and there is reason to believe that the individual components recorded in the amphibian are produced by activity of neurons analogous to those found in the cat medulla.

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† Nomenclature of Ecker and Wiedersheim (7) is used throughout.

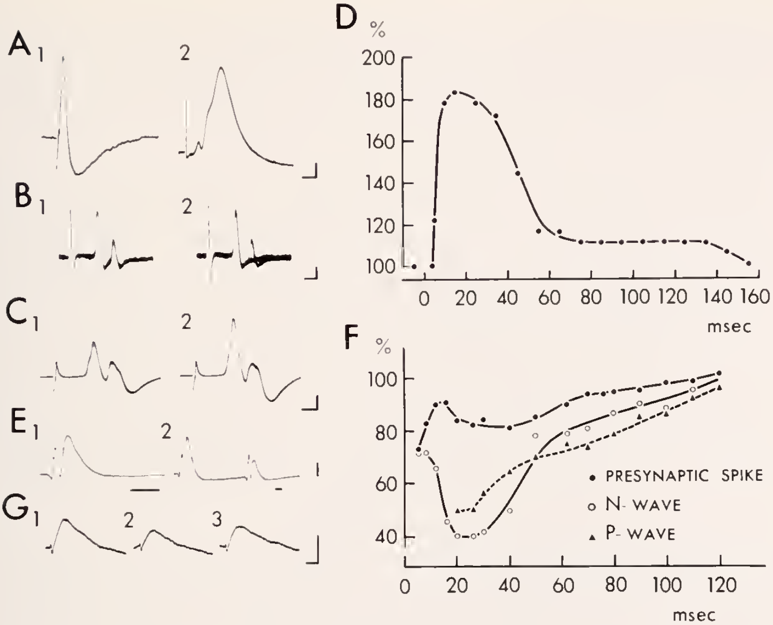

FIG. 1. A_1, DCN surface potential evoked by supramaximum single shock stimulation of ipsilateral cutaneous antibrachii and manus lateralis nerve (AML); monopolar recording electrode 1.2 mm caudal to obex, 0.3 mm from midline. A_2, same event, but recorded at a slower oscilloscope sweep speed and at a later time in the experiment. Calibration: vertical 20 μV, A_1 and A_2; horizontal 10 msec, A_1; 2 msec, A_2. B_1, superimposed antidromic potentials recorded from ipsilateral superficial tibial nerve after stimulation of DCN with cathodal pulses delivered through a micropipette placed 1.0 mm caudal to obex, 0.9 mm from midline. Stimulation rate 1 Hz. B_2, same at 10 Hz. Calibration: 0.1 mV, 1 msec. C, superimposed antidromic potentials recorded from ipsilateral cutaneous antibrachii lateralis posterior nerve (ALP) before (C_1) and 20 msec after (C_2) conditioning stimulation of AML, stimulating micropipette 0.9 mm caudal to obex, 0.3 mm from midline. Calibration: 0.2 mV, 0.5 msec. D, plot of the time course of the changes in excitability in the experiment illustrated by the specimen records in C. Ordinate: percentage change in amplitude of the direct antidromic potential. Abscissa: condition-test intervals. E, DCN surface potential evoked by brachialis longus superior nerve (BLS) volley (recording electrode 1.2 mm caudal to obex, 0.3 mm from midline) before (E_1) and 30 msec after (E_2) conditioning stimulation of brachialis longus inferior nerve (BLI). Calibration: 0.1 mV, 5 msec. F, plot of the time course of the interaction experiment illustrated in E. Ordinate: presynaptic spike (●) and N-wave (○): percentage change relative to control value (E_1, 100%); P-waves (▲): size of test P-wave determined by subtracting the amplitude of the control conditioning P-wave from the total response and that value compared to control test P-wave (E_1, 100%). Abscissa: condition-test intervals. G, thalamic focal potential produced by test volley in contralateral BLS before (G_1) and 50 (G_2) and 100 msec (G_3) after conditioning volley in contralateral BLI. Calibration: 50 μV, 20 msec.

The initial diphasic wave had both the appearance expected of a fiber tract compound action potential and a short latency consistent with the conduction delay expected from dorsal column axons (4, 5). This wave followed rapid stimulation rates (200–300 Hz) and most probably reflects the incoming volley in collaterals of primary afferent fibers ascending in the dorsal columns. The negative component (N-wave) ranged between 50–400 μV in amplitude and reached its peak 3.5–5.0 msec from the time of stimulation. On the basis of its latency, its sensitivity to rates of stimulation greater than 150–200 Hz and to asphyxia, it was most likely a synaptic potential, similar to the cat cuneate N-wave. In the latter situation the N-wave is apparently generated mainly by extracellular currents produced excitatory postsynaptic potentials at the first synapse of dorsal column axons in the medulla (9). The third component recorded from the surface of the medulla was positive (P-wave) and had a slower time course than the preceding two waves. The amplitude varied from experiment to experiment; the maximum was about 50 μV. In preparations with poor circulation it was sometimes absent. It was extremely sensitive to repetitive nerve volleys (complete eradication at 10–20 Hz) and to asphyxia.

Primary Afferent Depolarization: It has been assumed (9) that the P-wave is an indirect measure of depolarization of the presynaptic terminals of afferent fibers (primary afferent depolarization. PAD). However, we investigated PAD more directly in the frog DCN by measuring the excitability of a population of primary afferent nerve terminals to a brief stimulus applied through a microelectrode (Wall's technique, 10). When a submaximum cathodal current pulse was passed through an electrode appropriately placed in the DCN at the site of the largest N- and P-waves, a short latency antidromic spike followed by a series of potentials of lower amplitude was evoked in ipsilateral fore- and hind-limb cutaneous and mixed nerves (Fig. $1B_1$, C_2). From the latency measurements and the response to repetitive stimulation (Fig. $1B_2$) it seems likely that the early response is the result of direct stimulation of DCN nerve terminals by the current pulse and that the late response is either a synaptically produced reflex discharge from the same terminals (dorsal column reflex) (11) or a manifestation of the dorsal root reflex (12).

When the test current pulse through the microelectrode was preceded by a conditioning volley in a peripheral nerve, the excitability of the primary afferent nerve terminals in the DCN was enhanced. The enhanced amplitude of the directly elicited antidromic response indicates this (Fig. $1C_2$). In general, an increase in excitability indicates a depolarization of nerve terminals (15). The depolarization is responsible for the larger number of afferent fibers antidromically excited by the current pulse which then contributes to the compound antidromic spike.

The time course of the excitability changes in the experiment illustrated by the specimen records of Fig. $1C_1$ and C_2, is plotted in Fig. 1D. In this experiment maximum increases in excitability were found when the interval between the conditioning and test stimuli was about 15–20 msec. In some experiments the maximum change in excitability was produced at intervals up to 30–35 msec. The effect persisted for as long as 200 msec.

Inhibition of Synaptic Transmission: It is now generally believed (12, 13) that primary afferent depolarization leads to a diminution in the transmitter output from the depolarized terminals and therefore results in a depression of the excitatory synaptic actions evoked by these terminals (presynaptic inhibition) (12). And, indeed, we found that transmission at the first synapse in the frog DCN was correlated with the excitability of primary afferent terminals. Two different sets of experiments demonstrated this point. In one set of experiments it was shown that the N-wave, which is an easily recorded measure of excitation at this synapse (14), was depressed by preceding conditioning volleys in ipsilateral peripheral nerves (Fig. 1E). This depression had a time course (Fig. 1F) which paralleled that of the increased terminal excitability with a summit at condition-test intervals of 20–40 msec and a total duration of 150–200 msec. A parallel depression of the P-wave was seen in these experiments (Fig. 1F). In addition, the presynaptic spike was usually reduced in amplitude (Fig. 1F) and this, too, can be interpreted as an indication of depolarization of the presynaptic terminals.

More direct evidence for inhibition of synaptic transmission in the DCN was obtained in a second series of experiments which examined the effects of conditioning nerve volleys on the potentials evoked in the thalamus by peripheral nerve volleys. The extracellular field potentials produced in the contralateral thalamus by single peripheral nerve volleys consisted of a brief diphasic spike and a subsequent longer negative potential (Fig. $1G_1$). Preceding conditioning stimuli to the same or other peripheral nerves depressed the thalamic potential (Fig. $1G_{2,3}$) again with a time course paralleling that of the PAD produced by similar nerve volleys.

Discussion

Previous anatomic (3, 6, 14) and physiologic (4, 5) studies have indicated the presence in the amphibian of an afferent dorsal column pathway ending in the medulla. The present experiments demonstrate that primary afferents projecting to this area are capable of inducing primary afferent depolarization in this region. This conclusion is based on the presence of a positive surface wave (P-wave) recorded only over the surface of the DCN. The P-wave was evoked by peripheral nerve volleys which increased the excitability of primary afferent nerve terminals when tested with Wall's technique and which also produced a delayed reflex response of the same terminals. Furthermore, the time course both for the depression of synaptic excitation of second-order neurons in the nucleus measured by the N-wave and for transmission through the nucleus to the thalamus paralleled that of the increased excitability of afferent fibers. The parallelism between the time course of PAD and depression of synaptic events has been previously observed in various pathways within the amphibian and cat spinal cord (12, 13, 15) and within the cat cuneate nucleus (9, 11, 16); this suggests that a presynaptic inhibitory mechanism operates in the amphibian DCN. These evidences of presynaptic inhibition will have to be supported by definitive intracellular studies. These studies are necessary to rule out the presence of long-duration postsynaptic inhibitions or occlusive processes. However, it is assumed that intracellular recordings from cutaneous afferent preterminals would demonstrate depolarizations evoked by conditioning volleys.

The older conception that sensory pathways consisted of simple one-to-one relays of neurons conveying information in an unmodified form from the periphery to the more rostral areas of the central nervous system has been altered by findings showing that transmission in sensory pathways can be modified by many central and peripheral presynaptic influences (13). Presynaptic inhibition appears to be a major means for the selective processing of sensory information at the level of the first sensory input. It is of interest, therefore, that we are able to demonstrate PAD in the primitive amphibian homologue of the mammalian nucleus gracilis and cuneatus. Our results suggest that presynaptic inhibitory mechanisms were laid down early in phylogeny, and thus may represent a fundamental mechanism for the processing of sensory information both in simple and advanced vertebrates.

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The Multiple Sclerosis "Hot-Bath Test": A Putative Model in the Intact Frog*

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Introduction

It is well established that small increases in the body temperature of multiple sclerosis (MS) patients can worsen existing signs and symptoms as well as provoke the appearance of additional neurologic findings (1, 2). The latter forms the basis for the so-called "hot-bath test" which aids in the diagnosis of MS when the induced hyperthermia provokes the appearance of new findings that support a disseminated pathologic process (3).

Recent studies strongly support the idea that this temperature effect is caused by a reversible blocking of impulse conduction in demyelinated nerve fibers (4–6). The following mechanism has been suggested. Normally, the amount of action current generated by a nerve impulse is approximately 5 to 7 times in excess of the minimum amount needed merely to maintain conduction (7). If this safety factor is reduced to a level which just barely permits conduction to occur, the smallest additional insult could cause a conduction block even though a similar change would not block conduction in a normal nerve. In MS, it appears that some demyelinated nerve fibers traversing a plaque are in such a precarious state that a small temperature increase upsets this delicate balance in the direction of conduction blockade (6).

The increased temperature sensitivity of demyelinated nerve is not a specific effect (5). Any lesion which, like a demyelinating lesion, tends to depress axonal function without destroying the conducting membrane would be expected to cause a similar increased sensitivity to temperature. This has been demonstrated in excised nerves both for mechanically and thermally produced lesions (4, 5). In this paper the temperature sensitivity of electrolytically produced lesions was studied in the intact frog. Phenomenologically the results show a striking similarity to the clinical "hot-bath test" and present a putative model for further study.

Methods

Electrolytic lesions were made in intact frogs by passing a direct current between a #22 needle anode, insulated except at its tip, inserted percutaneously near the sciatic nerve in the mid-thigh of a frog (Rana pipiens) and a 3 × 4 cm plate cathode placed on the skin over

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the dorsal aspect of the lower trunk. The nerve was first located by adjusting the position of the percutaneous electrode until a very small cathodal stimulus applied at this electrode was able to elicit a contraction of the gastrocnemius muscle. Direct current from a Grass Model CCU1 constant current unit was then applied for two minutes and repeated if necessary at increasing intensities until there was minimal evidence for a lesion, judged by a mild paresis or slowness of movement of the involved leg. As expected from variables such as electrode placement and current shunting, there was considerable variation in the necessary current. Minimum effective currents ranged from 8 to 30 milliamps. Animals developing mild and stable motor abnormalities were selected for the following experiments.

Studies in the intact frog

Moving pictures were made of the frog jumping at room temperature (23–25°C) beginning 1 hour after making a lesion. These were compared with films made immediately after immersing both legs (up to the junction of legs and trunk) for two minutes in a beaker of water maintained at the stated elevated temperature. In this way the normal leg served as a control for the observed temperature effects.

In vitro studies

The involved sciatic nerve was dissected out of the frog and placed in a stimulating-recording apparatus designed to stimulate and record from opposite ends of the nerve while the temperature of the middle segment (which included the site where the percutaneous, lesion-producing anode had been positioned) was varied. Stimuli consisted of single supramaximal, 0.1 msec rectangular pulses from a Grass S-8 Stimulator and stimulus isolation unit. Compound action potentials were recorded monophasically by crushing the nerve between the recording electrodes. Potentials were displayed on a Tektronix Dual Beam 561 A Oscilloscope after amplification with a Tektronix FM 122-Low-Level Preamplifier and were photographed with a Polaroid camera. Details of the recording chamber and the method of temperature control have been described elsewhere (5).

Results

The frog shown in Figure 1 has an electrolytic lesion in the right leg. A 1–5 shows a leap that appears normal. However, note that at rest (A, 1) the posture of the legs is asymmetric, the right positioned lower than the left; there was also a mild but definite slowness in its movements. B, 1–5 shows a leap immediately after immersing both legs in 37°C water for two minutes. A paresis of the right leg only is clearly evident. Although the tissue temperature is unknown, bathing this animal for 15 minutes in water as low as 33°C still produced a definite paresis of the same leg. The normal leg does not demonstrate any change with these temperature changes. C, 1–5 shows a leap five minutes later; the paresis has almost completely reversed. Although the electrolytic lesion could involve muscle as well as nerve, the severity and widespread distribution of the paralysis favors the latter as being responsible for the phenomenon.

The sciatic nerve was dissected out of the animal and placed on the stimulating-recording apparatus described above. Note in Figure 2 the decrease in the compound action potential amplitude beginning at 28° with nearly complete block at 31°C. These effects are reversible. This represents an increased sensitivity to temperature as normal frog sciatic nerves did not begin to show changes until around 32° with nearly complete block occurring abound 34–36°C (5). Similar results were confirmed in five other frogs.

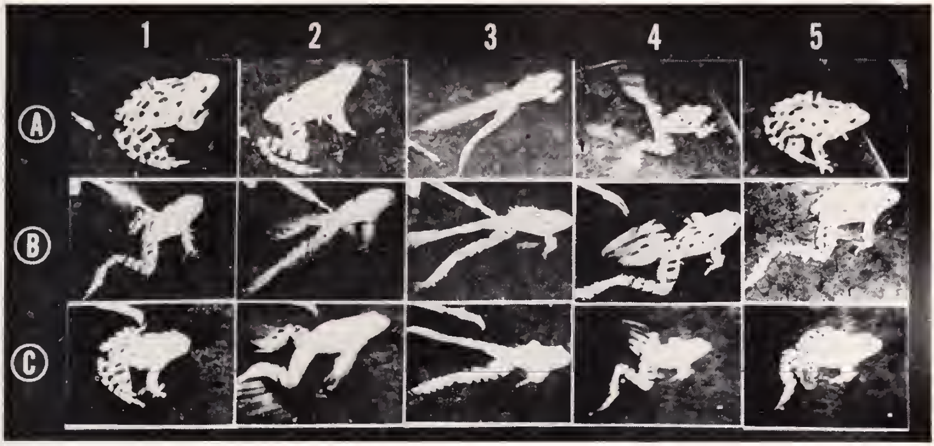

Fig. 1. An electrolytic lesion was made in the right leg of this animal as described in Methods. Following this the above sequences were filmed. A, 1–5 shows a leap at room temperature (25°C). In A 1 the affected leg is held slightly lower than the left but the leap is otherwise unremarkable. B, 1–5 is a leap in the same animal after immersing both legs in a beaker of 37°C. water for 2 minutes. Note that the right leg does not participate fully in the leap while the left leg movements are normal. After five minutes at room temperature the right leg paresis has almost completely reversed, C, 1–5. This temperature response was elicited numerous times in this same animal over a 3-day period.

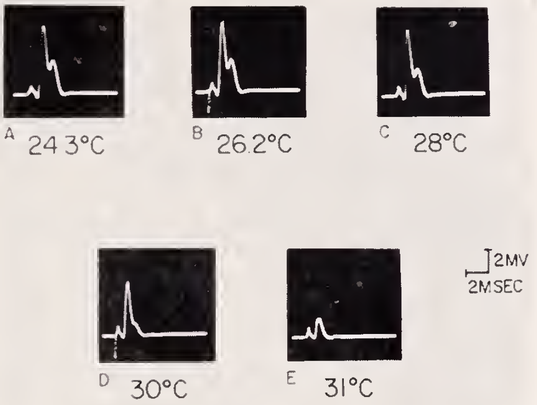

Fig. 2. In vitro effect of temperature on the sciatic nerve removed from the electrolytically injured leg of the frog depicted in Figure 1. The nerve blocks at lower temperatures than normal nerve. See text.

Discussion

This study demonstrates both in the intact animal and in vitro an increased susceptibility of electrolytically injured nerve to heat block. A similar phenomenon has previously been shown for pressure-injured (4, 5), thermally injured (4), and demyelinated nerve (5). This seemingly ubiquitous response of damaged and demyelinated nerve to temperature adds support to the hypothesis that the hyperthermia effect in MS is related to an alteration of conduction in diseased axons. It would appear that an increase in temperature can lower the conduction safety factor; and if the latter drops below a critical level, conduction suddenly ceases. These ideas do not indicate the precise mechanism by which temperature elevation depresses the safety factor. However, it is reasonable to consider that it may be related to alterations in specific parameters such as action potential amplitude and duration, excitation threshold, axoplasmic resistance, or nodal current density. These ideas have also been borne out in recent mathematical analyses of a model demyelinated nerve based on minor modifications of the Hodgkin-Huxley formulation of the nerve impulse (6). This model predicts that the blocking temperature is a steep function of the degree of demyelination. To put the results another way, given a population of fibers with varying degrees of demyelination, a mild hyperthermia will block conduction in those fibers whose blocking temperature is now exceeded by the increased body temperature. The model also predicts that at the same time that more fibers are blocked, the conduction velocity of those fibers which remain conducting is increased by increased temperature. Experiments on demyelinated peripheral nerves suggest the validity of these calculations (5, 8).

These relationships suggest that the clinical worsening with hyperthermia in MS is caused by a block of conduction alone and not by any change in conduction velocity. Since the low conduction velocity in demyelinated nerve increases with temperature elevation (6, 8), it should, if anything, tend to improve clinical function. That the net effect of hyperthermia is a clinical worsening is not surprising, considering that the conduction velocity change with a small change in body temperature probably would not exceed several meters/sec and is a minor effect compared to a complete block of conduction.

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Perceptual Conjunction of Disparate Sensory Stimuli*

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It is well known that there is an upper limit to the number of different stimuli a person is able to report correctly if they are presented within a small interval of time. For the normal adult this number is usually greater than two (1–3). However, in the case of young children and patients with certain types of cerebral disorder there is frequently an abridged perceptual response to multiple stimuli; and only one of two or more simultaneously presented stimuli will be reported (4, 5). It is for this reason that the method of double simultaneous stimulation (DSS) is of diagnostic value in neurology.

DSS pairs can include two qualitatively different stimuli in the same sense mode, e.g., making a clicking sound on one side of the patient's head and a ringing sound on the other side; or two similar stimuli can be applied to different regions of a sensory field as when touching two widely separated parts of the body; or the two applied stimuli can be in different modalities, e.g., a touch on one hand accompanied by a brief flash of light into one or both eyes.

The use of DSS for neurologic diagnosis was recommended by Oppenheim in 1885. Various investigators subsequently studied this phenomenon and their work has been summarized by M. B. Bender in his classic book *Disorders of Perception* (4). In this book and in a long series of papers Bender and his coworkers explored the applications of DSS and described the diverse response patterns exhibited by normal subjects and by patients with a variety of neurologic conditions. Though the technique of DSS is deceptively simple, the observed results are not easy to categorize and are even more difficult to explain.

For example, one productive use of DSS is to elicit focal sensory defects that are not demonstrable by methods of single or successive stimulation. Yet it cannot be inferred that occult sensory deficits underly all occlusive perceptual interaction effects. The well-known Bender Face-Hand Test provides a constant reminder that intact parts of a sensory field are systematically ignored when they are stimulated in certain DSS combinations. Furthermore, it is often the case that a patient who experiences a partial loss of somatic sensation on the side opposite his cerebral lesion will fail to show extinction or any other interaction phenomenon on DSS testing. Focal sensory abnormalities are thus neither necessary nor sufficient conditions for the occurrence of anomalous perceptual responses to DSS. This absence of a regular causal connection between them argues strongly against the proposition that clinical extinction depends on functional alterations in a sensory projection system (6, 7).

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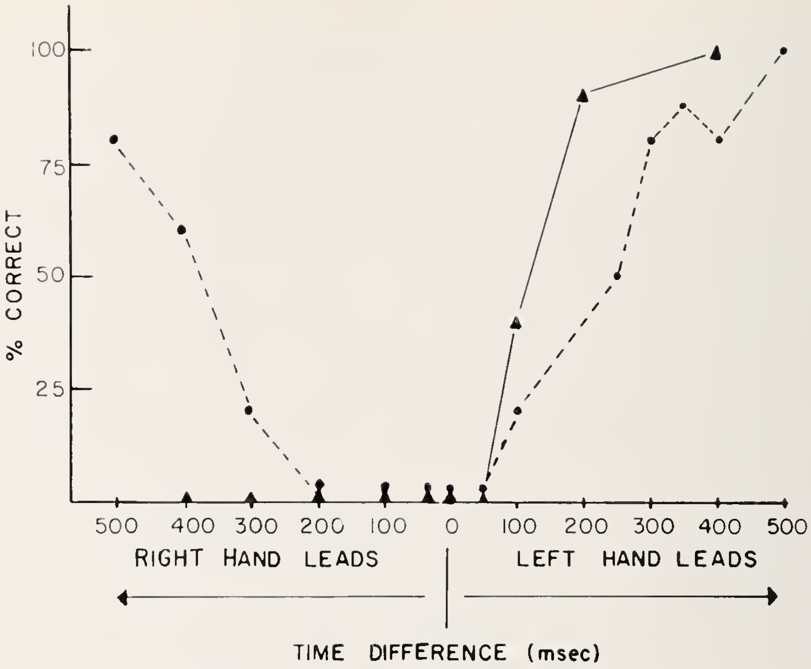

Fig. 1. Percent correct reports of two stimuli as a function of the time interval between them. DSS pair consisted of brief electrical stimuli applied through ring electrodes to the middle fingers of each hand. Intensity was adjusted so that either stimulus was always perceived when given alone, but when presented simultaneously, only the stimulus on the intact side was reported. Time intervals were randomized. Both subjects were patients with right cerebral lesions and left-sided sensory deficits. In addition to the prolonged time course of the interaction (more than 1 sec for patient ▲), note the prominent order effect (1958).

There are other compelling reasons for rejecting physiologic explanations of DSS interaction effects that posit destructive interference or suppression of neural activity at sites within an elementary sensory system. Among them are: 1) The time course of DSS interactions, which are often more than an order of magnitude longer than the latency of primary cortical response to stimulation (Fig. 1); and 2) the occurrence of intermodal interactions, including transmodal displacement of sensory stimuli, when the elements of a DSS pair involve different modalities of sensation (1, 8–10).

In this paper we will present and analyze another DSS response pattern whose occurrence has an important bearing on the plausibility of alternative theoretical explanations of DSS interaction effects. This particular response pattern involves the joining together of attributes of both stimuli of a DSS pair into the verbal report of a single combined perception. This type of interaction, anticipated by Bender (4), is a form of perceptual conjunction as distinguished from the phenomenon of transmodal displacement which it resembles to some extent.

The following five cases are illustrative of this phenomenon.

Case 1: Patient M.W.[1, 2]

1. e: now tell me what you feel (examiner lightly touches right side of patient's face and the top of his left hand).
   P: YOU TOUCHED MY FACE
2. e: tell me what you feel now—keep your eyes closed (touch right side of face; loud click near left ear)
   P: (RECOILS SUDDENLY, LOOKS STARTLED, PUTS HAND ON RIGHT CHEEK) YOU SLAPPED ME

The conjunction of stimulus qualities by the patient (the touch and the click) is quite apparent here, in both his verbal and his behavioral responses. In fact the illusion that he had been rudely "slapped" was so strong that the patient had to be reassured that he would not be hurt in further testing. It is conceivable that in this case the form of the examiner's query (asking the patient what he *felt*) may have contributed to the abnormal response pattern. However, this does not appear to be a factor in the second case, since the patient's response is unrelated to the language of the examiner. In this case the two stimuli are a touch and the sound of a finger snap.

Case 2: Patient I.L.

1. e: keep your eyes closed and tell me what you feel (touch right hand; loud snapping sound near left ear)
   P: I HEARD SOMETHING HERE (POINTS TO LEFT SIDE)
2. e: okay—what about now (pin prick on dorsum of right hand; finger snap near left ear)
   P: (OPENS EYES, STARTLES) THAT WAS A SHARP SOUND
3. e: did you feel me touch you
   P: NO

Here too, the behavioral component of the patient's reaction that accompanied her utterance was a striking feature of the response. However, the conjunction resulting from the two stimuli may be confined to the verbal report alone as in the following case.

Case 3: Patient E.E.

1. e: tell me where I touch you now (snap on left side; touch right hand)
   P: (OPENS EYES SLOWLY, NO VERBAL RESPONSE)
2. e: where did I touch you
   P: YOU TOUCHED ME WITH A SNAP

Although he was depressed, showed little spontaneity, and did not elaborate

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[1] The clinical details of the 4 reported cases are not important for the purposes of this paper. All four patients had some degree of organic mental defect: Cases 1 and 3 showed focal left-sided signs; Case 2 had deficits on her right side and Case 4 had no focal neurological findings. The normal subject was a 7-year-old boy.

[2] The format of these illustrations is similar to the one developed for reporting examinations of aphasiacs (11). All of the examiner's statements and actions are presented in lower case type; patient responses are capitalized. Nonverbal events are indicated by brackets.

his responses, this patient was alert and cooperative in the examination. He did not give incomplete or terse replies to questions except on DSS testing. The next patient provided a large number of interesting conjoined responses to heteromodal stimulation, two of which are included here.

Case 4: Patient M.G.

1. e: (click on left; touch right hand)
   P: YOU GAVE A HEAVY SNAP ON MY RIGHT HAND
2. e: what else
   P: AND A SMALL SNAP ON THE OTHER HAND
3. e: tell me what I do / tell me what happens / look here (light flash temporal field O.D.; click near left ear)
   P: A BIG ELECTRIC SHOCK ON THE RIGHT SIDE
4. e: anything else
   P: A SMALL ONE ON THIS SIDE (POINTS IN SPACE TO LEFT OF HEAD)

The next case illustrates the perceptual conjunctions of a sound and a touch by a normal young child.

Case 5: Subject C.D.

1. e: (finger snap upward on left; tough right hand)
   P: YOU SNAPPED YOUR FINGERS HERE (POINTS TO SIDE OF LEFT EAR)
2. e: anything else
   P: THEN YOU BROUGHT YOU HAND DOWN IN FRONT OF ME LIKE THIS (DESCRIBES AN ARC EXTENDING FROM LEFT SIDE OF HEAD ACROSS BODY TO LATERAL SIDE OF RIGHT THIGH)
3. e: did I touch your hand
   P: NO
4. e: did I touch you anywhere
   P: NO

This example shows clearly that failure to differentiate the touch as a separate stimulus event is linked to the construction of a simplifying hypothesis. The event was perceived as a gestalt organized around the finger snap. In all subsequent responses, when the situation was no longer novel, the subject correctly identified both elements of the heteromodal DSS pair separately.

The DSS defect illustrated by each of the five subjects in at least one of their response samples has several interesting characteristics:

1. The patient reports perception of a single stimulus event.
2. Properties of the unreported stimulus are actually incorporated into his verbal response. This results in the explicit report of a single percept having attributes of both stimuli.
3. The conjunction of stimulus qualities is exhibited in a number of different ways.

(a) addition of stimulus qualities: (Case 3)
(tough + finger snap)—"You *touched* me with a snap."

(b) modification of the report of one of the stimuli by adducing to it qualities of the other: (Case 2)
(pin prick + finger snap)—"That was a *sharp* sound."

(c) the report of a percept that corresponds to neither stimulus but possesses attributes of both: (Case 1, 4 and 5)
(clicking sound + touch)—"You slapped me."; ". . . you brought your hand down in front of me."

4. The patient's verbal report of a combined percept is frequently accompanied by appropriate psychomotor activity indicating that the verbally reported conjunction is a structured perceptual illusion and not a purely linguistic phenomenon.

5. In most cases the conjunction occurs only once or twice in a given test situation demonstrating that this behavior is in part related to the novelty of the stimulus pattern.

Discussion

Illusory conjunctions of diverse sensory stimuli are distortions of perception. As is the case in many other types of anomalous interaction effects, they cannot be explained by recourse to theories of physiological inhibition, structural disconnection or inattention. It may be argued that appropriate behavioral responses to both stimuli in a DSS pair can occur with verbal neglect of one of them because of a lesion that disconnects a "perceptual center" from a "language center" elsewhere in the brain. Disconnections of this kind have been proposed to explain a wide variety of disorders affecting language and perception (12), including "unilateral sensory neglect" (13). A disconnection theory, however, cannot account for the phenomena of illusory conjunction described above wherein a stimulus, which is not explicitly identified as such by the patient, is still contained implicitly in his verbal report.

It is clear then that distorted response patterns elicited by the method of DSS are not created *de novo* by the failure of a particular neural structure. Even though the detailed characteristics of a DSS response may be determined to some extent by the locus of a lesion (14), it remains questionable whether such interaction defects can develop under clinical conditions of testing without some impairment of or lapse of mental function. To posit and search for another gnostic center or another functionally specific neural tract in order to explain the common sensory illusions reported here is reminiscent of the "ether" construct in physics that proved to be superfluous (15).

Considerably more data, behavioral as well as physiological, are required before one can specify the neural mechanisms operating to produce DSS phenomenology. However, on the basis of existing facts it is possible to characterize these perceptual illusions in terms of the processes involved (16, 17). The value of this kind of intermediate, heuristic formulation is that it enables an ordering of the facts and identifies productive lines of research without reinforcing cumbersome assumptions required by premature anatomical and physiological speculation. Specifically, we propose that the illusory percepts elicited by DSS arise from an alteration in criteria for detecting subordinate stimulus elements as separate entities when they occur as ambiguous figures in a complex sensory event. This could be brought about either by overload of a system, especially one with reduced information handling capacity, or by a reduction in signal-noise ratio affecting the figure-ground relationships in multiple stimulus configurations. The failure to explicitly differentiate one stimulus in a DSS pair, but by conjunction to fit it into s simple hypothesis centering on the other stimulus, demonstrates more clearly than do other interaction effects this close relationship between DSS phenomena and general mental functions.

Acknowledgments

The authors are deeply indebted to Dr. Morris B. Bender who sent us out into the neurological world to test with two safety-pins instead of one and held his light high so that others could follow the trails he blazed.

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Some Novel Concepts of Cerebellar Physiology*

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The evidence accumulated over a century and a half of investigation is overwhelming that the cerebellum is primarily concerned with the motor control of skeletal muscles of the vertebrate animal. It is our intention here, however, to focus on some research which suggests that it might have a broader function and to discuss its role in the control of convulsive seizures, in vegetative functions, and in emotional behavior, and finally to briefly indicate the present trend in our research in cerebellar physiology.

It was Rolando (1) who, following acute cerebellar ablations, reported that the cerebellum was responsible for motor activity. Flourens (2) about 20 years later clearly showed, however, that it only exerted a regulatory influence on motor performance. There were a few ideas expressed in the 19th century that the cerebellum might have other roles to play but they tended to be ignored because of the overwhelming defects in motor performance which followed upon its acute or chronic ablation.

Experimental chronic epilepsy can be readily produced in the rat by the application of cobalt powder to the cerebral cortex (3). After a preliminary period of slow activity paroxysmal outbursts of sharp waves and some spikes are present, localized in the cobalt area, which reach a maximum amplitude in between three and four weeks, and at this time the animal usually shows clinical signs consisting of clonic convulsions of the opposite face, neck and forelimb. The discharging foci usually remain active for one to two months. During this time both photo- and audio-stimulation can activate the epileptic activity.

Stimulation of the cerebellum usually inhibited this activity followed often by facilitatory rebound. Occasionally, an activation effect was noted (4).

Total cerebellectomy in normal rats produced a tendency to slowing and synchronization of the electrocorticogram (ECG). Epileptic manifestations were enhanced both when cerebellectomy followed or preceded cobalt application. The response to sensory stimulation changed significantly in the cerebellectomized cobalt epileptic rat. While the cobalt epileptic rat with an intact cerebellum quickly recovered from the convulsions produced by prolonged sensory stimulation, the epileptic cerebellectomized rat showed striking aftereffects consisting of spike and sharp waves and both generalized and focal clinical seizures. The poststimulation effect in many cases lasted for more than 15 minutes. Such prolonged poststimulation effects were never seen in the animals with intact cerebelli.

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A method of cooling the dorsal surface of the cerebellum in the awake, unanesthetized rat was then devised. A thin sheet of platinum foil was applied over lobules VI, VII, and VIII and part of the foil was brought to the surface, and a small cubicle was fashioned into which dry ice would be placed. In the first days after cobalt application when the ECG did not yet show epileptic activity, cooling of the cerebellum provoked generalized slow waves. It appeared 5–6 minutes after the cooling had begun. During the development of the epileptic activity following cobalt powder application when sharp wave and spikes were present, cooling of the cerebellum provoked facilitation of spiking with the appearance of a clonus of the forelimb and irradiation of the paroxysmal activity resulting in repeated generalized seizures. Figure 1 shows the effect of temporary cooling of the dorsal cerebellar surface on the development of cobalt epilepsy 8 days after cobalt application on the right frontal cortex.

This inhibitory influence is also demonstrated in records of unitary activity from the cerebral cortex and cerebellar cortex when simultaneously recorded during convulsions produced in acute experiments (5). Remarkable reciprocal relationships were shown to exist between the unitary activity of the cerebrum and cerebellum in various phases of certain convulsive states. We believe the cerebellum may have an important role in the prevention of and termination of cerebral seizures. Since these observations, which were reported about 10 years ago, the results have been confirmed and extended in our own and many other laboratories and cobalt has assumed an important role in the production of chronic experimental focal epilepsy in the mouse, rat, cat, rabbit, and monkey.

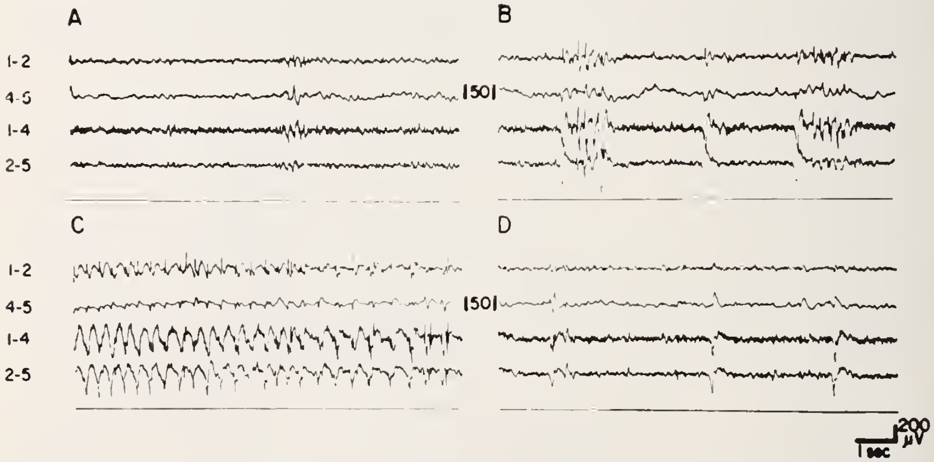


Fig. 1. Effect of temporary cooling of the dorsal cerebellar surface on the development of cobalt epilepsy in rat #93, 8 days after cobalt application on the right frontal cortex. *A:* before cooling, control record. *B:* 5 min. after the beginning of the cooling showing an increase in the spiking. *C:* 16 min. after the beginning of the cooling during the first of two spontaneous seizures; a second occurred 15 min. after the cooling was stopped. *D:* 55 min. after the end of the cooling period with a return to activity more nearly like that in A, although occasional clonic activity was still seen. The gain of channel 2 is increased. Control of temperature changes induced in the cerebellum by similar cooling at the time of sacrifice under pentobarbital anesthesia showed that the temperature of the cerebellum 3.5 mm below the surface dropped from 35.25°C to 30°C within 3 min. of the application of dry ice and to 27°C in 15 min (EEG & Clin Neurophysiol 14:383–398, 1962).

When cobalt is introduced into the amygdala or hippocampus in the cat, a more chronic focus results than is seen in this species after neocortical application (6–9). Stimulation of the cerebellar vermis during an epileptic attack limited to the rhinencephalon blocked the seizure in most cases. If the epileptic discharges had propagated to the neocortex, cerebellar stimulation would only effectively inhibit the attack if carried out during the clonic phase. This may explain the equivocal results in our laboratory of a cerebellar influence on neocortical-induced seizures both in the cat and monkey (10, 11).

Cerebellar ablation has been shown not to change barbital withdrawal convulsions in dogs (12). Hyperoxic seizures in rats were enhanced by cerebellar ablation and cerebellar stimulation usually produced some inhibition and rarely facilitory effects (13). Stimulation of the Pyramis Lobe VIII and Paramedius Lobe VIII H inhibits penicillin-induced seizures in lightly anesthetized cats and lesions in these areas produced an augmentation of spike frequency in the same preparations (14). Inhibition of cobalt frontal lobe seizures by implantation of cobalt on the cerebellum of the rat has been reported (15) but the work could not be confirmed in the cat (16).

The above work seems to indicate that it is the cerebellar vermis (fastigial nuclear reticular formation system) which is concerned in this mechanism rather than the hemisphere (dentate thalamic outflow), but this cannot be certain at this time.

Some observations which are of interest is the effect of diphenylhydantoin on the electrical activity of Purkinje cells (17–19). It has been shown that the discharge frequency of Purkinje cells is raised remarkably in doses not much greater than is used clinically in humans. When DPH was given at dosages of 10 mg/Kg intravenously in cats the spontaneous activity was changed from random variation in discharge frequency to a sustained high frequency discharge which persisted for several hours (Figs. 2 and 3). When cerebellar ablation either followed or preceded DPH administration, the effect of Dilantin® injections on cortical epilepsy bursts was reduced as compared to intact animals. When DPH was not given, there was a correlation between the frequency of the Purkinje cell discharge and the cortical discharge (Figure 4). In the treated animals these fluctuations were not seen. The Purkinje cell discharge remained uniformly rapid. Under these conditions phenobarbital behaved like Dilantin but Tridione® and Diamox® did not, either in their effect on the

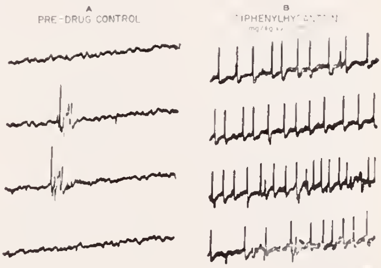


FIG. 2. Spontaneous Purkinje cell discharge recorded from the same cell in anterior cerebellar vermis before and after DPH (10 mg/Kg i.v.). Each trace is 100 msec in duration and is read from left to right, bottom to top. Negative is upwards. (A) Prior to DPH, spontaneous P-cell discharge rate is low with this particular unit discharging at about 5/sec. A waveform similar to the climbing fiber response follows each somatic spike. (B) One hour following DPH, spontaneous P-cell discharge had increased to approximately 140/sec (Life Sciences 10:575–582, 1971).

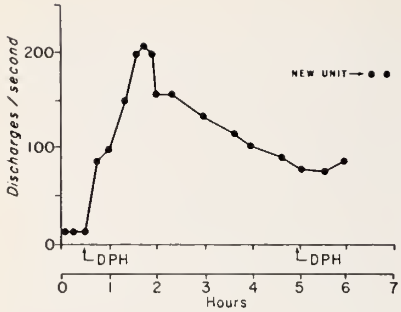


FIG. 3. Average discharge frequency of a typical Purkinje cell before and after diphenylhydantoin (10 mg/Kg i.v.) injected at the time indicated by the arrow below the abscissa. Prior to DPH, the P-cell was discharging spontaneously at 15 Hz. One hour after DPH, discharge frequency had increased to 210 Hz and 5 hours after DPH, discharge was still maintained at 90 Hz. A second dose of DPH was administered but the unit was lost at this point. Other units discharging at 160–200 Hz could be easily located. (Life Sciences 10:575–582, 1971.)

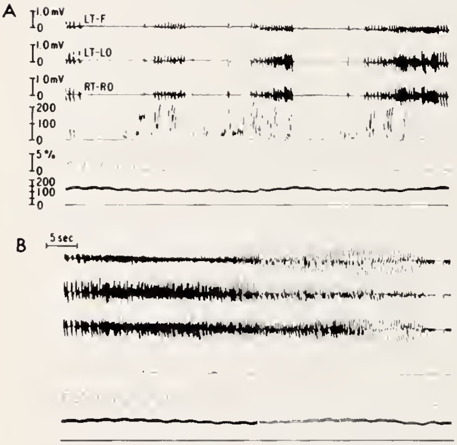


FIG. 4. Oscillograph record of cerebellar P-cell discharge and cerebral cortical seizure activity in a cat with penicillin-induced epileptogenic focus. The bottom six traces (B) are continuous in time with the upper six traces. Each trace and its calibration represents (from top to bottom): 1. Electrocorticographic (ECG) activity recorded between left temporal cortex and an indifferent lead in frontal sinus. 2. ECG activity recorded between left temporal and left occipital cortex. 3. ECG activity recorded between right temporal and right occipital cortex. 4. Discharge frequency of cerebellar P-cell located in the anterior vermis calibrated in discharges/sec. 5. End-tidal CO_2 calibrated from 0–5%. 6. Arterial blood pressure in mm Hg. *A* illustrates bilaterally recorded bursts of epileptiform activity and its relation to P-cell discharge activity. Toward the end of *A* the burst becomes a continuous long-lasting bilaterally recorded seizure discharge (B). Note the relative absence of P-cell discharge after the first few seconds of continuous seizure activity (Life Sciences 10:575–582, 1971).

penicillin foci or on the cerebellar activity (Figure 5). These same authors also reported on electroshock convulsions in mice with congenital malformation of the cerebellum (stagger mice) compared to their normal litter mates. Seizure thresholds were identical in the stagger mice and their litter mates. When supramaximal shocks were used, it took significantly more Dilantin to prevent seizures in the stagger mice with no cerebellum compared to their normal litter mates with intact cerebelli.

Clinical application of these observations have only recently been attempted but within the last year electrodes have been implanted on the anterior lobe of the cerebellum in eight patients. Beneficial effects of this cerebellar stimulation in patients with intractable psychomotor seizures have been reported (20).

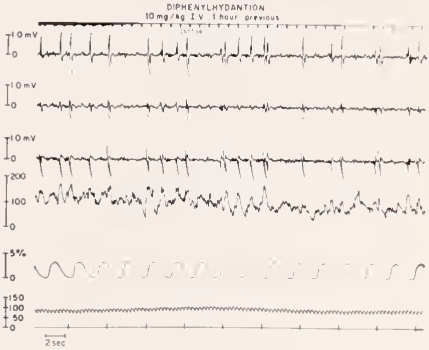


FIG. 5. Cerebral cortical seizure activity and cerebellar Purkinje cell discharge following administration of diphenylhydantoin to a cat with an epileptogenic focus. This record was obtained three hours after subpial injection of penicillin into anterior sigmoid gyrus and one hour after administration of diphenylhydantoin (10 mg/Kg i.v.). The eight traces and their calibrations represent: 1. A time marker with a deflection at one second intervals. 2. ECG activity recorded from left temporal cortex to an indifferent lead in frontal sinus. 3. ECG activity recorded between left temporal and left occipital cortex. 4. ECG activity recorded between right temporal and right occipital cortex. 5. Average discharge frequency of the cerebellar P-cell under study, calibrated in discharges/second. 6. End-tidal CO_2 calibrated in % of expired gas. 7. Arterial blood pressure in mm Hb. 8. Blood pressure baseline. Note the change from intermittent burst to intermittent spike activity corresponding with sustained high frequency discharge rates recorded from Purkinje cell (Life Sciences 10:575–582, 1971.)

There are numerous experiments which have demonstrated beyond doubt that stimulation and/or ablation of the cerebellum can influence a host of visceral functions (21–26). Induced blood pressure changes (27–30), sham rage (31, 32), the electrical activity of the hypothalamus (33–35), respiratory rate (29, 30, 36, 37), glucose tolerance curves (38, 39), pupillary changes (40, 31), motion sickness (41), bladder reflexes (42, 43), galvanic skin reflexes (44), changes in the duration of gestation (45) and of intrauterine pressure (23), and penile erection (46) are examples.

Both afferent and efferent connectors from visceral nerves and visceral centers of the brain stem and hypothalamus have been mapped by evoked potential studies by a number of workers (46–50). The demonstration of a cerebellar influence on vegetative functions of all types makes more understandable its possible role in emotional behavior.

Recent attention to the possibility that the cerebellum may influence emotional and behavioral states has been stimulated by the writing of Prescott (51, 52). The behavioral disorders which are found in many species of animals reared in isolation consist of movement stereotypes. Rocking, head-banging, self-clutching and self-stimulation, and hyperactivity are seen early; and as the animal matures into adolescence, violent aggressive behavior patterns including self-destructive biting have been described. It is Prescott's contention that maternal-social deprivation in isolation rearing of mammals represents a special case of sensory deprivation involving the somato-sensory system. Because the cerebellum is a part of the brain which is still developing while these abnormal behaviors are being produced and because it is an important part of the somatosensory system, Prescott has collected a number of observations in support of his theory that cerebellar maldevelopment induced by this kind of deprivation is in some way linked to these behavioral abnormalities: Among these are the following observations—1) it has been shown that an important outflow from the cerebellum goes to the limbic system. This originates from the cerebellar vermis and the floccular nodular lobe and, as indicated above, has recently been studied by evoked potential methods in dogs (46); 2) the cerebellum, the limbic system and the whole cerebral cortex are deficient in DNA in isolation reared animals as compared to normals (53); 3) aberrant electrical discharges in both limbic system and cerebellum in Harlow wire-cage, isolation reared rhesus monkeys have been described (54).

Electrical stimulation of the cerebellum in the unanesthetized cat causes, in addition to stereotyped motor responses, some behavior which appear related to emotional responses (55, 56). The animal cringes as though in fright at times and at other times a kneading of the claws in a pleasure-like reaction as a rebound following stimulation was seen. Stimulation of the midline vermis and fastigial nucleus caused a hypersensitivity to sound and touch and animals often attempted escape (57). Cerebellar ablations have resulted in persistent pleasure reactions such as constant purring and kneading of the claws while lesions of the fastigial nucleus resulted in lethargy (42, 58, 59). Squirrel monkeys who showed aggressive cage behavior before surgery became tame and more tractable following lesions of the vermis (60, 61).

Dr. A. J. Berman has made very convincing movies of alterations in behavior following two types of cerebellar lesions (62). He received from the Wisconsin Regional Primate Research Center two rhesus monkeys raised in partial social deprivation. These animals had become violently aggressive toward each other and toward humans. In each instance the surgery dramatically changed their behavior. They no longer fought when placed in the same cage. The effect lasted three months in the neocerebellar ablation but has persisted for over two years in the one with a vermian lesion.

Six infant monkeys were reared singly in wire cages from birth. They could see and hear other monkeys but had no physical contact with them. All six demonstrated the emotional and behavioral disorders typical of maternal-social deprivation. Two were operated on at 10 months of age. In one, the lesion involved the pyramis, uvula, flocculo-nodular lobe, paramedian lobe, culmen, and part of the lobus simplex. In the other the cortex of the neocerebellum was removed. The latter had the greater neurologic defect but both had recovered in four weeks. The six infants, two operated and four nonoperated, were placed in a large observation chamber for one hour once a month. This has gone on for over 2 years. The four nonoperated monkeys have persisted in their abnormal behavior, remaining huddled in their corner, rocking incessantly, and showing no interest in their surroundings. In contrast the operated monkeys after two months began to interact with each other. They moved freely about the cage, climbed the walls and began to touch and nip at one another. These differences between the operated and nonoperated controls have persisted.

Finally, six highly aggressive feral animals had been kept in Dr. Berman's laboratory for 2½ years and had been dropped from ongoing experiments because they could not be handled. One was operated on with a lesion involving the vermis with a good taming effect. Another with a neocerebellar lesion showed no effect. Of the remaining four intermediate degrees of taming were seen and this seemed to be in relation to the completeness of a lesion which should involve the paleocerebellum and the flocculo-nodular lobe. These effects are not due to motor defects. The neocerebellar lesions produced the greatest motor defect but had the least taming effect. None of these animals has been sacrificed as yet for neurohistologic investigations.

Before accepting this theory, it is important to obtain histological evidence of some modification of cerebellar structure in the abnormal socially deprived animals. This has not been done as yet. However, Professor János Szentágothai (63), using quantitative methods of which space will not permit a description here, has offered evidence that the vermian areas of the anterior lobe receiving the direct mossy fiber projection from the limbs can be modified by decreasing sensory input by confining the limbs in weighted wax boots. In the animals where movement has been prevented the molecular fibers only attain half the length of those in the controls. This suggests that the cerebellum could well be the site of some kind of histologic anatomic change in the animal suffering from the syndrome of maternal deprivation if the proper methods of study are employed.

The midline cerebellar areas including the vermian cerebellar cortex, the flocculo-nodular lobe, and the fastigial nucleus are the parts of the cerebellum which influence seizures, vegetative function or aggressive behavior. Do other parts of the cerebellum also have a new role to play?

Thirty years ago it was my belief that once the cerebellum of higher primates and man could be studied experimentally, the cortico-ponto-cerebellar-neodentate thalamo-cortical system would be found to assume a greater role in motor control in man than it exerts in subprimates. This was anticipated because of the extraordinary increased size of this part of the cerebellum in man as compared with all subhuman primates except the anthropoid apes. However, with careful analysis of motor effects of lesions carefully restricted to this lateral part of the cerebellum in primates (64–66) the classic signs of cerebellar ataxia usually noted in cage behavior following many cerebellar lesions simply do not follow destructive lesions here as long as they are entirely restricted to this newest cerebellar system. With the introduction of stereotaxic surgery to the dentate system in man by a large number of neurosurgeons, no classic signs of cerebellar deficiency follows purely dentate lesions (67–72). On the other hand, in the cooling experiments of Brooks (73), very specific motor defects were induced in monkeys trained to execute a specific movement. The cooling probably involved the whole dentate system and was not restricted to its most lateral parts which become so large and foliated in anthropoid primates and man. Brooks and his coworkers placed a cooling probe stereotaxically close to the dentate nucleus. This temporarily and reversibly inactivated the dentate when its temperature was reduced to 10° Centigrade. When nonvisually guided movement was attempted during cooling, performance deteriorated. But if during cooling the animal is allowed to guide the arm movements by vision, such errors in velocity control are modified and little or no defect can be measured.

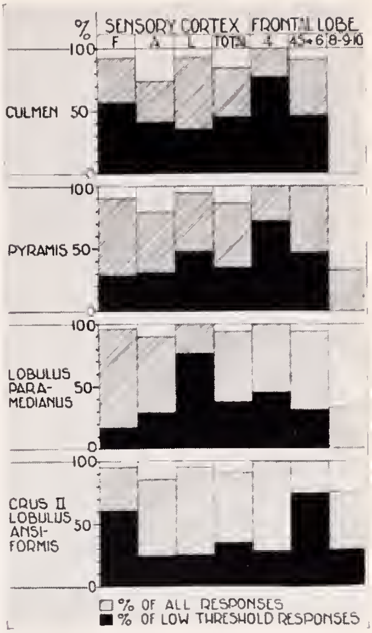
This study provides a critical observation on dentate function. It suggests that certain kinds of motor control are abolished with loss of the hemispheral system, but the idea of the task, its initiation, and its termination remain unaffected. And the important observation is made that vision will compensate for losses in this system, an observation which may account for the minimal effects in patients with hemispheral lesions.

The dentate nucleus of man and the anthropoid apes has essential differences from the nucleus in lower primates and subprimate species. This is based upon embryologic and histologic evidence. The nucleus in man and anthropoid apes consists of two parts, a dorsomedial microgyric, magnocellular older part, which is homologous to the dentate nucleus of lower forms (74) and a very much expanded new part which comprises the bulk of the dentate nucleus in man and the higher apes, the ventro-lateral macrogyric parvi-cellular part. These two parts differ in regard to cell types found (75, 76); in regard to iron reaction (76–79); embryologically (75, 80, 81); myelogenetically (82); and under pathologic conditions (74, 80, 83, 84).

One must next ask why this system reached such extraordinary size in man if it is not concerned with the traditional control of motor activities. It completely overshadows the older parts of the cerebellum present in all subhuman forms. An expanded role beyond that which we associate with the simple control of the rate, range and force of muscular contractions as observed in routine animal performance may eventually be found for this part of the human cerebellum. This extreme phylogenic development is not related to the cerebral cortex as a whole but specifically to the development of the frontal and temporal association areas whose projection to the pons in man as the fronto- and temporopontine fibers makes up one-fifth of the whole of the internal capsule. In a study of crossed cerebellar atrophy it was found that lesions of the sensory motor cortex produced selective atrophy of the phylogenetically older dorsomedian part of the dentate nucleus while parieto-temporal lesions which are mainly association areas were associated with atrophy of the newer ventrolateral part (74).

The most lateral portions of the cerebellum are differentially connected to these association areas as shown by evoked potential studies (85). A method of stimulation was used which was so gross and nonselective that it failed to show conclusive evidence of somatotopic relations found later to exist between face, forearm and leg areas of the cerebral cortex and their respective areas of the anterior lobe of the cerebellum (86) and demonstrated tremendous overlap between these somatotopic divisions (Fig. 6). However, with this crude method using strong stimuli capable of affecting large aggregates of cerebrocortical neurones clear distinctions were shown when the responses of the medial, intermediate and lateral zones of the cerebellum were compared following stimulation of area 4, areas 4 S and 6 and frontal association areas respectively (Fig. 6). Crus II lobulus ansiform (H VIII b) represents that part of the primate cerebellum which reaches such enormous size in man. It showed evoked responses when over 75% of all points in the frontal association area were

Fig. 6. Chart showing the presence and relative strength of responses from stimulation of topographical and cyto-architectonic subdivisions of the cerebral cortex when leading from culmen, pyramis, lobulus paramedianus and Crus II lobulus ansiformis. Data given in percentage of the total points stimulated in all the experiments. Note very little differences in total responses (cross-hatching) except in areas 8-9-10. The solid black shows percentage of + + + responses (responses of low threshold and high amplitude). When the percentages of low threshold or high amplitude responses are compared, differences are detected. Note differences between lobulus paramedianus and lateral part of the culmen in respect to topographical subdivisions and the greater percentage of + + + responses from face than either arm or leg in the Crus II lobulus ansiformus. In comparing the various cyto-architectonic areas of the frontal lobe, the greater percentage of + + + responses in vermian lobes from stimuli in area 4, while stimuli in area 6 and the frontal association area show the greatest percentage of + + + responses in the lobulus anisformis (J Neurophysiol 5:121–136, 1942).



stimulated at maximal strength and 25% of all points stimulated gave responses when minimal stimulus strength was used. This is strikingly different when compared to Culmen and Pyramis (IV & V & VII) in the medial zone and the lobulus paramedian (H VIII) in the intermediate zone. Here no low threshold responses were found when stimulating in the frontal association areas and either none or only 25% of stimuli of maximal intensity gave responses in the medial or intermediate zone lobules. The same tendency for low threshold responses to reach only the lateral zone was found when the temporal and parietal association areas were stimulated.

If lesions or cooling of the dentate nucleus alone are not productive of the classical signs of cerebellar ataxia what methods can one employ to unravel the functions of this part of the cerebellum so large in man and so selectively related to the association areas of the cerebral cortex. A study of single units in awake primates trained to perform stereotyped movements may help (87–89). While these investigations are only in their early stages, they hold great promise of providing us with information not available from the more artificial kinds of electrical responses to a volley of impulses given in a sledgehammer way to an aggregate of neurones or fibers.

My associates, Dr. Robert Grimm and Dr. Donald Rushmer, at the Laboratory of Neurophysiology of Good Samaritan Hospital & Medical Center (90), have recorded from dentate and interpositus nuclear cells during the performance of a patterned movement. They are asking: "Is the cerebellum, in particular its hemispheral parts, a place where we store away motor patterns once they are learned?" They have elected to study a repeatable complex movement

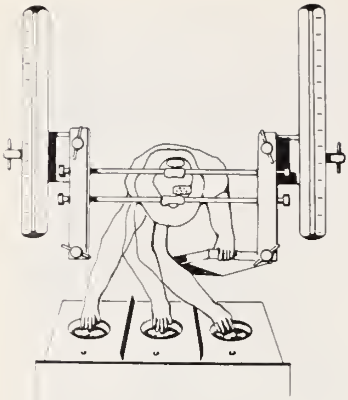


Fig. 7. Diagram of the experimental set up for experiments of cerebellar nuclear activity in patterned movements in primates (see text for further description. (ANA Transactions, 1973)

which links together many muscles in an explicit order. They have trained squirrel monkeys to touch three buttons in a row for a hypothalamic self-stimulus reward, a powerful reinforcer in this situation. The animal will perform this sequential patterned movement thousands of times during an experiment. The idea is to record from a single nuclear cell during many repetitions of the patterned movement to study its particular involvement statistically. The monkeys are allowed to play these buttons with their own style and their own tempo. Once the pattern is learned and the monkey is playing the pattern three times or more per minute, single unit recording is begun. Although the hypothalamic reward stimulus does evoke a detectable alteration in unit behavior after the task performance is ended, all measured effects of the hypothalamic stimulations have subsided before the tone signaling the next movement pattern is given.

Figure 7 illustrates the experimental set-up. Looking down at the chaired monkey from above, we see the headplug, fixed by pins to the stereotaxic frame, and carrying the hypothalamic electrodes. The silastic window over the occiput is the site for microelectrode penetration to the cerebellum. Upon a signal, the monkey reaches out with his left arm and touches the buttons in order from left to right, flexing and extending the arm twice after the initial ballistic movement. After touching button 3, the hypothalamic stimulus is given. Monkeys individually vary in their performance speed, but the actual motor pattern takes between 700–1000 msec. Some data on performance stability are shown in Figure 7. The records are from one well-studied monkey. The monkey's performance, given the degree of performance freedom, is remarkably uniform.

(A) is a raster-type display of 100 performances in sequence accumulated over about 20 minutes. Prior to this record, this monkey had played the buttons correctly about 22,000 times in the preceding months of training. The dots indicate the time between button 1 and button 2, and button 1 and button 3 for each performance. This is plotted to give an idea of the regularity of performance during data collection from a single nuclear cell, in this case cell B in track 70. Figures B and C, from the same monkey, evaluate performance stability over a period of months. Arbitrarily, performances from every tenth day were plotted. In B, note the extreme variability of elapsed time between the "go" signal and contact with button 1. Analysis of motion picture films reveals that the initial ballistic movement from rest to button 1 is of the same order of constancy as arm flight times between buttons 1 and 2. It is in the latency to the ballistic movement onset, and not in the performance per se, where variability occurs.

They have recorded from several hundred units in dentate and interpositus nuclei of four monkeys. Vertical tracks through these nuclei in performing squirrel monkeys yield two types of units as yet poorly separated: tonically active units, which modulate firing rates up or down, and "burst" units. As a class, tonic units appear similar to those described in the macaque by Thach (87, 88). Burst units have irregular firing patterns interrupted by bursts of spikes lasting 10–50 msec. It is the activity of units with this burst characteristic which they have been studying.

Burst units do not appear to be randomly located within the nuclear volume. They are grouped in certain areas of the nucleus in question and have been called "collectives." Burst units within these responding "collectives" having firing patterns correlated to specific epochs of time within the 700–1000 msec button pushing performance. The arm movements of flexing and extension to reach each button are somewhat similar. The units of the "collectives," however, were found to be involved with only one part of the performance, which suggests that these units are involved in the overall pattern and not in a particular muscle sequence per se. Figure 8 illustrates a track through the dentate nucleus ipsilateral to the performing arm which demonstrates the idea of a "collective". The dots indicate where individual burst units were located; filled dots indicate neurones firing in correlation with performance. Dotted dots indicate neurones with plus-minus correlation and open circles indicate units with no correlation with performance. This data was accumulated in a 3- to 4-hour recording session in which 1200 to 1500 performances are done, permitting analysis of 8–10 cells, each studied over about 100 performances. Let us now consider histogram correlations of burst unit activity and movement (Fig. 9).

Histogram arrays of spike occurrence for these cells with respect to each

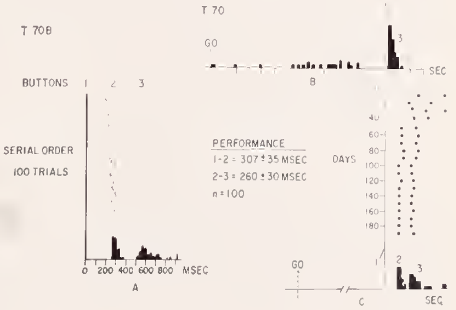


Fig. 8. Data on performance stability in pattern movement experiments in the squirrel monkey. A is a raster-type display accumulated over about 20 minutes. B & C from the same monkey evaluate performance stability over a period of months (see text for further description). (ANA Transactions, 1973)

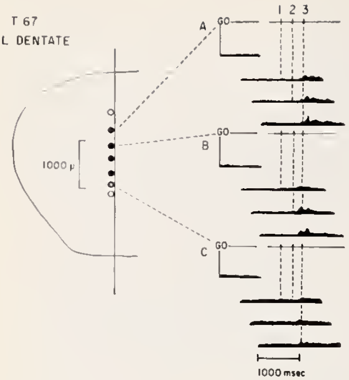


Fig. 9. Illustrates the responses of 4 cells in one tract through a "collective" in the dentate nucleus as an illustrative experiment collected over 3 to 4 hours during which 1200 to 1500 performances were done (see text for further description). (ANA Transactions, 1973)

button played are shown to the right in this slide. An array of 4 histograms is given for each unit. The top histogram in the array shows correlation of spike occurrences with presentation of the "go" signal. They refer to this as post-go event histogram.

The next histogram in the array covers the activities of the same unit but later in time, and from a different reference point. In this case, they compute the cross-correlation of spike occurrence 1000 msec before and after button 1 is touched. This same procedure is followed for buttons 2 and 3 in the next two histograms. In this way, as the monkey performs his task, telling them where he is on his own "program"—via button touches—they compute and display the spike occurrence with reference to specific, but arbitrary, points in the performance.

Units of this "collective" altered their discharge after the button 2 touch. The buildup in spike occurrence begins during arm flight from button 2 to button 3. The sharpest correlation of burst correlation is seen with respect to button 3.

The next unit in the track, (B) is located 300 μ below Unit A in the same track and responds during the same time epoch of the performance.

The lower unit is on the margin of this "collective" and shows the correlation with this portion of the performance to be fading.

This particular group of related cells, thought to be in ipsilateral dentate, is associated in time either with events shortly before or after the temporal epoch around button 2. This particular dentate "collective," as they have been called, is not involved with initiation of the pattern or with the phasic back and forth arm movement. It does, however, correlate with something going on as the performance ends.

A given "collective" itself appears physiologically and anatomically stable within the nucleus. We know this because, after a specific "collective" is identified, the area can be approached 10 days later and burst units with similar histograms can be retrieved.

"Collectives" of responding burst units in a pattern performance, reveal a stability in time and place, and become active during specific epochs of the motor pattern. With one or two exceptions to date, burst units are activated late in the performance, invariably after the initial ballistic movement is completed.

Participating burst units are not slaves to a single muscle's action and, quite likely, they are probably not involved in subunits of movement composition such as arm antagonists during flexion-extension oscillations between buttons. This is suggested because the onset and duration of discharge alterations bear little phasic relationship to such movements. What they do find are "collectives" of burst neurones altering their activities during some specific epoch of the pattern such as in the example chosen around button 3. It is their more or less irregular appearance in a sequence of performances, and their identity with specific parts of the pattern per se, and not to a specific muscle sequence, that seems the most important observation.

Speculation concerning the significance of these findings, viz., the origin of the burst unit response, how "collectives" come into existence, and this mosaic model for assembling a pattern movement by utilizing different "collectives" are all under study by our group at this time. The burst response may be due to axon collaterals of climbing fibers with destination to Purkinje cells in the ipsilateral cerebellar hemisphere. At this point all that can be concluded is that they have uncovered units in the dentate and interpositus nucleus which have a characteristic and consistent relationship to a patterned sequence of learned movement rather than to the movement itself. What happens if, while recording from a unit in an established "collective," a change in the movement pattern is ordered, or if the monkey is simply asked to play the original pattern slower or faster are, in addition to the study of the origin of the burst responses, now under study.

Conclusion

We have yet to learn how the cerebellum performs to control the somatic motor system, where its most important role lies. Perhaps unit recording in trained awake animals with computer analysis of the results may give us this information in due time. Regardless of how it performs its unique function, its role must be expanded to other than simply motor control. Something important seems to happen to convulsive phenomena, to a variety of vegetative functions, to emotional behavior and perhaps even to the highest level of intellectual function when a cerebellar influence is introduced into the nervous system. Whatever it does to modify the activity of other parts of the brain, it probably does the same thing in all its possible varied roles. Its uniformity of structure throughout vertebrates and within its various divisions in higher mammals makes this most likely.

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Pathogenicity of Mokola, a Rabies-Related Virus, in Baby Mice*

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Introduction

Mokola virus is a member of the Rhabdo group of viruses and has been shown to be serologically and morphologically related to rabies (1). The virus was originally isolated in Ibadan in 1968 from pooled viscera of shrews (Crocidura spp) (2) inoculated into newborn mice (1). There have been two known human infections of this virus, one of which was fatal (3, 4). Histologic sections of human brain from which Mokola virus was isolated and stained with phloxine tartrazine showed large numbers of finely granular cytoplasmic inclusion bodies in many neurons (3). This finding was found to be unique to this rabies-related virus. So far as can be ascertained a comparative pathogenicity of mouse-adapted and nonadapted Mokola virus has never been reported. This paper presents data on the pathogenesis of lesions induced by Mokola virus (mouse-brain-adapted and nonadapted) in newborn mouse brain, and shows that the unique inclusion bodies observed in the human brain can be reproduced in mouse brain as a basis for diagnosis.

Materials and Methods

Animals: Inbred Swiss albino mice maintained in this laboratory were used. They were between 2–3 days of age at time of inoculation.

Viruses: The fifth baby mouse-brain-passaged Mokola virus (designated P_5), and the original Mokola-infected human brain (3) (designated P_0), and a second mouse-brain-passaged rabies virus were used in these studies. They have all been stored at −70°C for at least six months. Ten percent suspensions of each virus containing brain were made in phosphate-buffered saline containing 0.75% bovine albumin (bovine albumin, fraction V). Virus (0.02 ml per dilution) was inoculated intracerebrally into 2–3 day-old mice. Titrations were carried out in ten-fold dilutions in newborn mice. Infectivity end points were determined by the method of Reed and Muench (5).

For pathogenetic studies the virus inoculated into each mouse was titrated and found not to be more than 1 $\log_{10}$. Normal uninoculated, and animals inoculated with 10% mouse brain served as controls. Inoculated animals were killed daily (4 per day/group) and brains removed, washed to remove blood and stored at −70°C for virus titration (pool of 3 brains). Those for histology were placed in Rossman's fixative. Sections were stained with hematoxylin and eosin and phloxine tartrazine and examined microscopically.

Complement-fixation (CF) test: The modified micro CF test as carried out in this laboratory has been described (6). Immune mouse ascitic fluids were inactivated at 56°C for 30 minutes before use. Results are expressed as the reciprocals of antigen dilutions.

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Results

Summary of results of histopathologic findings, infectivity titers, and CF antigen titers are shown in Table I. These are based on the average of results from two experiments.

Histopathology: The nonadapted Mokola virus (original infected human brain—P_0) did not induce any spectacular changes in inoculated mice during the first two days after inoculation. During this period none of the virus inoculated mice showed any signs of sickness. Most of the effects on the infected mouse brain were evident by the fifth day postinoculation. These changes include perivascular cuffing, mostly by neutrophil polymorphonuclear leucocytes and lymphocytes. There was sparse infiltration of glial tissue by degenerative polymorphonuclear cells. These lesions were most marked in the mid-brain. At this stage no "inclusion bodies" were observed. By day six

TABLE I

Brain Histopathology of Mokola Virus (P_0 and P_5) Infection in Mice

Days PI	Histopathology		Titers*			
			P_0		P_5	
	P_0 Unadapted (human brain)	P_5 (Adapted Mouse brain)	Infectivity $\log_{10}$ (LD$_{50}$)	CF*	Infectivity $\log_{10}$ (LD)$_{50}$	CF*
1	No abnormality	No abnormality	0	0	0	0
2	Mild focal congestion	Mild congestion	0	0	5.2	0
3	Moderate generalized congestion	Mild congestion and early perivascular cuffing. Focal "leucocytosis"	5.5	0	6.6	64
4	Moderate generalized congestion	Moderate generalized congestion. No cuffing.	≥ 7.3	16	6.4	16
5	Generalized congestion, mild perivascular cuffing by polymorphs and lymphocytes; sparse infiltration of glial tissue by degenerative polymorphs. Lesions most marked in midbrain. No inclusion bodies seen.	Marked congestion, no cuffing, no inclusion bodies.	≥ 7.3	8	6.6	16
6–9	Generalized congestion, perivascular cuffing, focal necrosis midbrain. Intranuclear inclusion bodies in neurons.	N.D.	7.1	8	N.D.	N.D.

0 = Negative; N.D. = Not Done;

P_0 = initial titer 2.5 ($\log_{10}$)

P_5 = initial titer 4.0 ($\log_{10}$)

* Expressed as reciprocals of dilutions (based on pool of 3 mouse brains each).

postinoculation and continuing through day 9 postinoculation most of those changes observed on day 5 were still present, but in addition large numbers of granular intranuclear inclusion bodies, perivascular cuffing, and neuronal degeneration were prominent. Infected animals did not show any gross evidence of encephalitis clinically during the period of infection until death. The average survival time of these P_0 inoculated animals was 9 days.

Mouse brain adapted Mokola virus (P_5) did not induce severe encephalitis histologically as shown by unpassaged Mokola virus (P_0). No inclusion bodies were seen at any time in P_5 inoculated mouse brains. These animals, however, showed severe illness by the third day postinoculation, and all were dead by the fifth day postinoculation.

The histology of rabies-infected mouse-brain harvested five days postinoculation showed typical Negri bodies mostly in the neurons of the mid-brain. Other changes observed were perivascular cuffing microglial proliferation and neuronal degenerative changes.

Virus and CF titers: No virus or CF antigen was detected in the brains of mice inoculated with the unpassaged (P_0) Mokola virus within the first 48 hours postinoculation. By day 3 postinoculation, however, virus in the brain was as high as 5.5 $\log_{10}$, which increased to 7 $\log_{10}$ within 24 hours (i.e., by day 4 postinoculation). This level was maintained till the end of the experiment (i.e., 9 days postinoculation). CF antigen was not detected till day 4 postinoculation at which time a peak titer of 16 was obtained.

In contrast to the above, animals inoculated with mouse brain adapted Mokola virus (P_5) showed high virus level within 24 hours postinoculation. The peak of virus titer observed three days postinoculation was 6.6 $\log_{10}$ and remained at this level until day 5 when all the animals were moribund. Virus infectivity titers in this group of animals were very much below those reported for the P_0 inoculated animals. The CF titer produced by P_5 inoculated animals within 3 days of infection was as high as 64, or four times the amount produced by P_0 inoculated animals four days postinoculation.

Discussion

Minimal changes observed in the brains of animals inoculated with the mouse-brain-adapted Mokola virus (P_5) suggest relative intrinsic resistance of the brain cells to the effects of the virus. The resistance is reflected by low virus titer, although with short incubation period. The early deaths of this group of animals, however, requires a different explanation. One can speculate that the mild brain reaction results from differences in invasiveness of the virus (in comparison to the unpassaged virus) and is not a reflection of the amount of virus inoculated, since the amount of virus inoculated into each mouse was not more than one $\log_{10}$. Early death in these animals may be due to tissue antigen-antibody reaction, as suggested by the histopathology, although this remains to be investigated. It is anomalous, though, that animals inoculated with unadapted virus produce more virus, show more severe reaction in the brain cells, and yet live longer than those inoculated with mouse-brain-adapted virus. It is obvious from the different effects of each virus on mouse brain tissue that infection and brain-cell resistance are dependent on the nature of the virus.

The CF antigen produced by each virus indicates that its level is not dependent on the amount of virus produced. Thus P_5 Mokola virus although producing less infectious virus, nevertheless has the most CF antigen. This suggests a soluble antigen, probably nucleoprotein in nature. Indeed the antigenic relationship of Mokola virus to rabies has been shown to be closer by CF test than by neutralization test (1). There is a strong possibility that the CF antigen or rabies and all rabies-related viruses is nucleoprotein and common to all.

The inclusion bodies observed in the brain cells of mice inoculated with P_0 virus are similar morphologically to those reported by Familusi et al (3) for the fatal human case. There is however one important distinguishing difference in that those observed in human brain were cytoplasmic, those in the mouse brain were intranuclear and again not cytoplasmic as in rabies. The reason for the differences in the locations of these bodies in man and mice is yet to be studied. Like rabies, Mokola virus in the mouse brain failed to show any inclusion bodies after about 3 or more passages in this tissue. The inclusion bodies described for Mokola and demonstrable readily by phloxine tartrazine stain might be unique to this virus and could be a simple method of distinguishing this virus from street rabies.

Summary

The pathogenesis of lesions induced by Mokola, a rabies-related virus, mouse-brain-adapted and nonadapted strains were compared. Mouse-brain-adapted Mokola virus did not induce as severe an encephalitis as the unadapted virus. Higher virus titers, with less CF antigen, and longer survival time were shown by the unpassaged virus. In addition, granular intranuclear inclusion bodies were seen in many cells where neuronal degeneration were prominent only in the unadapted virus. These differences in the two strains of virus were attributed to differences in invasiveness and other properties and not to the amount of virus inoculated. The intranuclear inclusion bodies are unlike Negri bodies of rabies and can be an aid in distinguishing Mokola virus from rabies. CF antigen of Mokola virus is considered to be nucleoprotein and common to other rabies related viruses.

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Transient Selective Amnesia for Merchandise Prices

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This paper reports the cases of two merchants who experienced a transient amnesia for merchandise prices. The phenomenon may be considered as an aspect of the syndrome originally described by Bender in 1956 (1) and more extensively in a subsequent communication (2). A preliminary note has appeared in the Brazilian literature (3).

Case 1: W.D., a 72-year-old, Russian-born, married, cloth storekeeper in Rio de Janeiro awoke on the morning of July 1, 1963 complaining of a severe headache. He obtained relief, as he had in the past, from aspirin; and the following morning he felt well and went as usual to his store. As his first customer entered, he realized that he was unable to recall what to charge for the purchase and had to summon an employee. He abstained from selling and engaged himself trying to remember the prices of items in sight. He was unsuccessful and decided to call his daughter at her home but was unable to recall either her number or that of his son. He then realized that he had forgotten the birthdates of his children, the current year, and other familiar dates. He now was thoroughly frightened. He went home for lunch and mentioned his trouble to his wife who considered him otherwise quite normal in behavior. After a half-hour rest he returned to his shop and after a few hours his memory had returned to its usual state. Neurologic examination was unremarkable. Blood pressure was 150/100 and routine laboratory studies were normal. The patient was able to recall everything that had happened during his period of memory loss and to evaluate critically his feelings and course of thought.

Case 2: H.S., a 61-year-old, Russian-born, married, cloth merchant opened his business as usual on the morning of September 14, 1967. He first examined several bills of sale and 15 minutes later, when he tried to evoke the price of a certain cloth he was unable to do so. He tried to think of various prices but was uncertain as to the correct ones. He could easily read all tagged prices. He became worried but did not tell anyone and ordered his employee to make a detailed price list. He tried to call his home but had to look up the number in the directory after which he could dial. He then asked his wife to come down to the store. While waiting for her he read a newspaper without difficulty. He kept trying systematically to recall the prices but "all were rubbed out as by an eraser." Two hours after his trouble began he began to improve and, in a short time, recollection was perfect. As in the first case, his subsequent recall of what had occurred was intact and neurologic examination was unremarkable. As of the present (April 1973) there have been no recurrences in either case.

Discussion

These cases resemble those reported by Bender (1, 2) and Fisher and Adams (4) and Patten (5) in that they involve a brief episode of memory loss without confabulation in an elderly person, in the absence of demonstrable neurologic disease and without recurrence over the years. They differ from Bender's patients in that neither man was disoriented or confused and was able later to recall all events that had occurred during the period of memory loss. My patients and Bender's were also alike in that they were aware of and concerned with the loss of recall during the acute episode.

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An outstanding feature in my patients was the selectivity; in that the main symptom was the inability to recall prices of stock. In a sense, this is amazing, for a small shopkeeper's survival in Rio de Janeiro depends on his ability to immediately quote such figures. Prior to Bender's paper, the memory loss might have been regarded as psychogenic, but neither man was experiencing any business or personal problem at the time. Also, there are now numerous instances of highly selective losses of memory occurring in patients with undoubted brain damage. Weinstein, Marvin, and Keller (6) found that soldiers who had sustained severe brain injuries frequently had an enduring selective amnesia for aspects of military service—such as inability to remember the work they did and the rank they held. A man who aspired to a career as a professional baseball player was concerned because he could not remember who had won the league batting championship. Similarly, Janis (7) noted that after electroshock, memory loss may be limited to certain areas of personal identity.

It is evident that both in Brazil and the United States, the nature of transient amnesia in its several varieties, remains a mystery.

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Appearance of Signs and Symptoms of Multiple Sclerosis in Response to Cold

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That change in body temperature may be associated with change in the signs or symptoms of patients with multiple sclerosis has been a most intriguing aspect of this illness. With temperature elevation, new signs may appear or signs already present may worsen—only to remit with a return of body temperature to normal (1–6). This phenomenon is the basis of a clinical diagnostic test: the "hot bath" test. A suspected diagnosis of multiple sclerosis may be confirmed if upon the induction of hyperthermia additional neurologic signs appear which fulfill the requirement of anatomic dissemination. Symptoms may even fluctuate during the day in association with the circadian temperature rhythm; visual acuity (7), motor power, and coordination (8) have been shown to worsen and improve with the normal rise and fall in temperature. Less well known is the improvement of symptoms which may occur with reduction of body temperature below normal (7, 9, 10). Unfortunately, such improvement lasts only as long as the body temperature remains below normal, so that the therapeutic use of cool baths and cold packs is of limited value.

The patient whose case report follows was diagnosed as having multiple sclerosis. She is of interest in that among her original complaints was the appearance or worsening of symptoms on exposure to cold weather.

Case Report

The patient was 36 years old when first examined. She had for three years labile hypertension treated with chlorthiazide and potassium chloride.

For four years she had been having fleeting paresthesias of both legs and pain in both shoulders. For three years she complained of intermittent lightheadedness and vertigo not associated with change in position. For two years there had been a mild but progressive weakness and dragging of the right leg. She described a "floating" sensation in this limb when she walked. Climbing stairs and arising from the floor had become difficult and she thought that under these circumstances both legs were weak. When reaching the top of a flight of stairs she had to pull herself up by the bannister. She further described some degree of unsteadiness when walking, occasionally colliding with objects. She had to give up dancing because her feet felt as though "they were glued to the floor." One year before her examination she experienced electric shock-like pain in the lower half of the right face involving the nose, malar area, and chin which was of a transient nature but occurred several times each day. Two weeks before she was seen, similar shock-like pain was felt on the left side of the neck and radiated to the left shoulder and down the arm to the elbow. She had urinary urgency but not incontinence.

The motor symptoms worsened when she was exposed to cold weather. She described increased difficulty walking, a stiff gait, and again the feeling that her legs felt glued to the

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floor. In addition, there were involuntary "jerky" movements of all limbs, even when at rest. These all returned to what for her was normal when she warmed herself. On one occasion she went from a theatre into the cold street and suddenly could not walk. She had to be carried to her car.

At the time of her first neurologic examination she had a wiggling gait but was not otherwise unsteady. She sometimes wavered slightly from side to side on tandem walking. Although she could walk on her heels and toes she became more unstable and required support. Individual muscle strength was normal. Toe tapping of the right foot was considerably slowed. Reflexes were 3+ bilaterally and plantar responses were normal. There were no abnormalities of sensation or cranial nerve function. Testing of visual fields with a 2 mm red object was normal. Her blood pressure was 135/95.

The following laboratory studies were normal: CBC, ESR, serum VDRL, lupus preps, two-hour postprandial serum glucose, serum sodium, potassium, chloride and bicarbonate, urine VMA, urine catecholamines, and nor-metanephrine, and chest x-ray.

She was hospitalized one month after the first examination. One week before hospitalization she had had an episode of diplopia in the horizontal plane which lasted for five days. There were additional findings at the time of her second examinaton. She now had a fine bilateral finger-to-nose and heel-to-knee dystaxia and was unsteady on tandem walking. Reflexes were very brisk and there was unsustained left ankle clonus. Babinski responses remained absent. Sensory and cranial nerve examination were normal. The following laboratory studies were done and were normal: CBC, urinalysis, urine porphobilinogen, and serum glucose. Serum sodium was 139 mEq/L, potassium 3.8 mEq/L, chloride 98 mEq/L, SGOT 21, SGPT 14, CPK 2 sigma units (normal). A second serum potassium was 4.0 mEq/L. EKG was within normal limits; x-ray of the chest was normal; x-ray of the cervical spine showed small hypertrophic spurs involving the posterior aspects of the bodies of C^3 and C^4. A spinal tap was done; the opening pressure was 150 mm. The fluid was clear and colorless. Manometrics performed in the flexed, neutral and extended positions were normal. Spinal fluid gamma globulin was 17.5% (normal 5–20%). The CSF protein was 31 mg %, sugar 67 mg %. There were 18 polymorphonuclear leukocytes. Cold water caloric responses were normal.

A hot-bath test was performed and after a rise of body temperature to 103.5°F. there was nystagmus on upward gaze and, on lateral gaze, a bilateral MLF syndrome with nystagmus of the abducting eye and complete inability to maintain the adducting eye beyond the central position. The abducting eye tended to return to the neutral position. The next day the patient was examined in a walk-in refrigerator in an attempt to reproduce the symptoms she complained of when exposed to cold. The temperature of the refrigerator was 35°F. and the patient was wearing only her pajamas. She developed a slow, labored, rigid and unsteady gait and needed assistance in walking and also developed a marked sustension tremor and an increase in finger-to-nose dystaxia bilaterally. Within a few minutes of exposure to normal room temperature, those symptoms induced by hypothermia disappeared. Six months later, in order to film her response to cold, she was exposed to an ambient outdoor temperature of 29°F. Her gait by this time had become mildly spastic and careful, with a tendency to fall to the right and unsteadiness on turning. Tandem walking was also performed slowly, and she sometimes fell to the right. Diadochokinetic movements were rapidly done but she incompletely supinated the hands. There was a slight terminal tremor on the left on finger-to-nose testing. Her signs worsened considerably on exposure to cold. She seemed to become rigid, barely able to walk, indeed, as if her feet were glued to the ground. She could not tandem-walk. Diadochokinetic movements were also slow and laborious, taking one second to go from pronation to supination. She tended to wing-beat on the right, had a sustension tremor of both arms, and bilateral finger-to-nose dystaxia with a wide amplitude tremor. Because of shivering, she was unable to keep a thermometer in her mouth, so her body temperature at this time was not obtained. Serum sodium and potassium were drawn at this time and remained normal.

Discussion

Alteration of symptoms and signs in multiple sclerosis in response to cold has been sparsely reported in the clinical literature. Simons (2) questioned twenty-one multiple sclerosis patients about the effects of heat and of cold upon symptoms which were already present. Strength was reported to have worsened in fourteen percent, spasticity in twenty-four percent, numbness in twenty-nine percent, and bladder symptoms in twenty-nine percent of patients during cold weather. He did not describe the appearance of any new symptoms.

Examination of patients on exposure to cold has revealed worsening of signs in only two cases. Watson (9), in a study of the effects of lowered body temperature induced by exposure to cold air or immersion in cold water on eight patients with multiple sclerosis, reports one case in whom, paradoxically, there was worsening of a spastic paraparesis. However, this patient's temperature was not reduced but rose to 99.9°F. He suggests that those cases of Simons' with worsening of symptoms during cold weather might have resulted from overdressing, overheating indoors, or actual elevation of body temperature above normal. Namerow's (7) patient with multiple sclerosis and a central scotoma, whose visual acuity fluctuated during the day, was also subjected to cold. His visual acuity improved as body temperature dropped but precipitously deteriorated when he began to shiver and his body temperature rose.

The patient reported here is unusual in that her early complaints were in response to cold and she in fact had both a worsening of signs and a development of new signs both to heat and cold. She developed a bilateral medial longitudinal fasciculus syndrome and upward nystagmus in response to a hot bath test but when subjected to cold she developed a rigid-ataxic gait, severe enough almost to prevent her from walking, a bilateral intention tremor which was not present prior to the examination, and marked worsening of a mild finger to nose dystaxia. There are no other cases reported, to my knowledge, describing this dual response to both hot and cold. It is possible that the rigid-ataxic gait might also have been produced during the hot-bath test but this type of total body immersion makes certain parts of an examination difficult. Immersion of one or more limbs in hot water (3) would have been a preferable method, raising total body temperature while allowing for ease of examination. Although shivering and subsequent rise in body temperature cannot be completely dismissed in this patient, the possibility remains that the signs produced were a result of cold itself.

The responses to temperature fluctuations should be further investigated, and, furthermore, physicians should question and examine patients' responses both to heat and cold.

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Neurogenic Disorders Affecting Muscle: Five-Year Analysis of Muscle Biopsies

I. Segmental Atrophy*

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Introduction

The classic histologic concept of neurogenic disease of muscle has been that "segmental atrophy" is the major diagnostic feature (1–4). Biopsy interpretation of neurogenic atrophy has been predominantly based on the fact that, following experimental denervation, the muscle fibers appropriate to the affected motor unit undergo atrophy without structural change.

Most previous studies have dealt with retrospective analysis of biopsy material with full knowledge of the clinical diagnosis (1–6). For this reason, a prospective study of neurogenic disorders of muscle was undertaken (7). This study represents a five-year summary of the pathologic features of neurogenic disease of muscle encountered in that study.

Materials and Methods

172 patients with clinical evidence of neurogenic disease had 265 muscle biopsies over a five-year period.

Clinical assessment of patients preceded and was independent of biopsy analysis. The patients were divided into 3 clinical groups: myelopathy (78 cases), peripheral neuropathy (85 cases) and a mixed group (9 cases) (8). The criteria for clinical diagnosis is described elsewhere in detail (8–10). Most of the patients clinically diagnosed as myelopathy, had findings compatible with diffuse involvement of the spinal cord, such as amyotrophic lateral sclerosis and progressive spinal muscular atrophy. All patients with the clinical diagnosis of peripheral neuropathy had polyneuropathies. Polyneuropathies were not clinically differentiated from polyradiculopathies and both were considered as part of the neuropathy group unless associated evidence for spinal cord disease was also present. The mixed group contained clinical features of both the spinal cord and the peripheral groups. The various associated illnesses included diabetes mellitus, alcoholism and nutritional deficiencies, infections, collagen diseases, or other systemic disease. Laboratory studies included electromyography, serum enzymes, blood chemistries, urinary excretion of creatine and creatinine and appropriate x-ray studies.

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These histologic features were systematically recorded without regard to the clinical diagnostic impression.

Muscle fiber-size was calculated in cross section utilizing the method of orthogonal diameter measurement (11).

Histograms were made for each fiber-size pattern. A representative muscle was utilized for each histogram shown (deltoid, tibialis anterior, or quadriceps femoris). Muscle fiber-size was plotted in 10 micron increments as the abscissae and the appropriate percentage of the 200 measured fibers plotted as the ordinate. Criteria for normal muscle have been previously established (7). Abnormally small fibers were those which on cross-section measured less than 25 micra in the major axis diameter, and were present in more than 1% of the total fiber population. Three patterns of abnormally small fibers were regularly encountered: large groups contained more than 10 adjacent abnormally small fibers; small groups in which 10 or less adjacent abnormally small fibers were found; and a random distribution of abnormally small fibers. Representative microscopic fields of each of these patterns were selected and 200 fibers were measured in successive concentric planes from the center of the selected field. A calibrated monocular Zeiss eye piece with a synchronized, freely movable, manually operated micrometer was used; and all fiber measurements were made with a 40× Zeiss Planacromatic objective.

Biopsies from 137 patients with varying myopathic disorders were also available for comparative analysis.

Results

Muscle Fiber-Size

1. Normal muscle biopsy from clinically unaffected patients biopsied because of suspicion of systemic vasculitis (fig. 1 a,b,c)—34 patients served as the

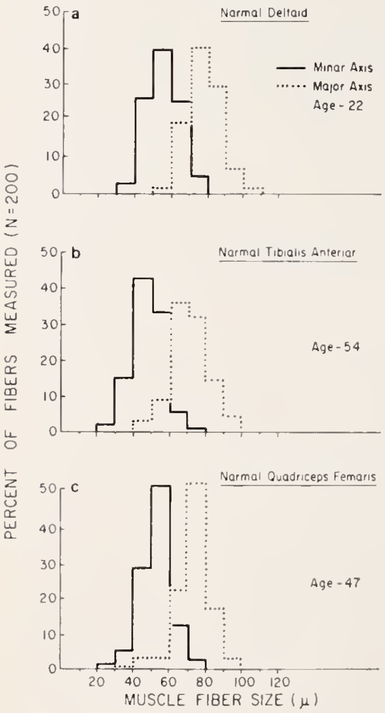

FIG. 1. Normal muscle biopsy from patients with neurogenic disorders of muscle. Each axis shows a normal distribution pattern. a–Deltoid; b–Tibialis Anterior, c–Quadriceps Femoris.

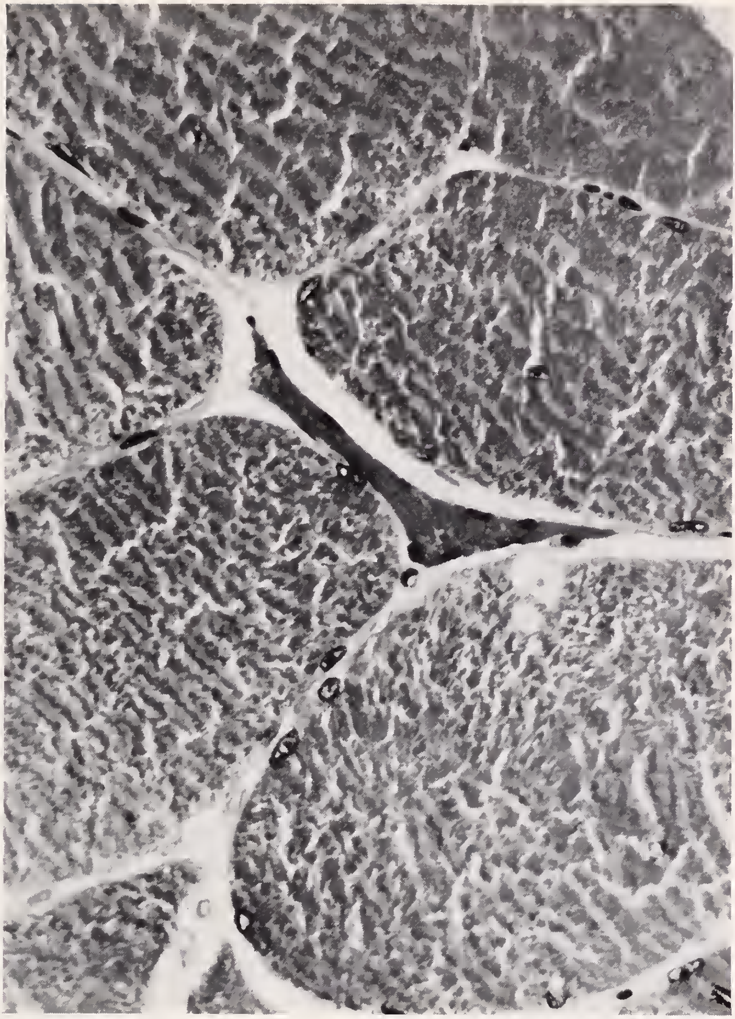

FIG. 2. Random distribution of abnormally small muscle fibers, approximately × 700.

control against which the other groups were contrasted. This pattern parallels that of normal muscle in which the usual bimodal size frequency distribution of major and minor axis diameters was obtained (11). In the three muscles examined (deltoid, tibialis anterior, and quadriceps femoris), the results were similar. No minor axis diameter was less than 25 micra and no major axis diameter was greater than 108 micra.

2. Random distribution of abnormally small fibers (Figs. 2 and 3)—30 patients. The pattern demonstrated in this group showed two changes which differentiated it from the group with normal biopsies. The range of fiber-size was greater, varying from 5 to 78 micra in the minor axis diameters and from 8 to 118 micra in the major axis diameters. The mean of each axis decreased corresponding to the presence of abnormally small fibers. The peak incidence,

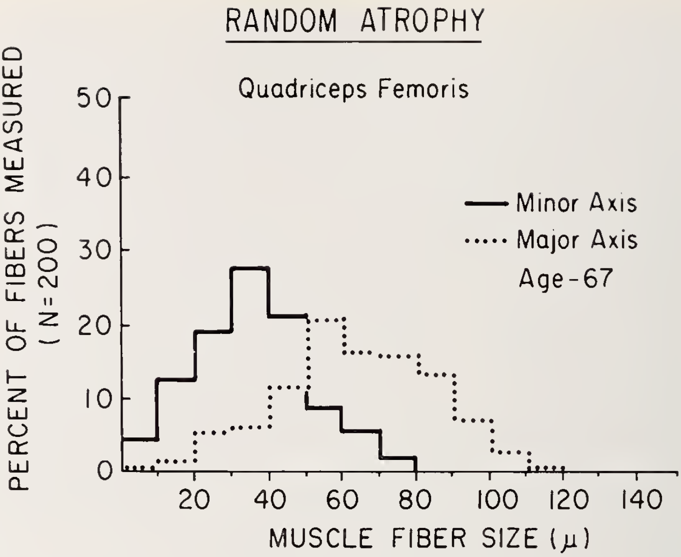

Fig. 3. Random atrophy—the distribution of fiber size is wider than normal; and the peak incidence of each axis shows a shift toward the smaller fiber size.

though well defined, was certainly not as sharply demonstrated as in the patients with normal muscle biopsy.

3. Small groups of abnormally small fibers (Figs. 4 and 5)—46 patients. The muscle bundles containing small groups of atrophied fibers showed further variability. The pattern (shown in a single muscle group in Figure 5b) differs from that of the random group only in the greater frequency of abnormally small fibers. In the example shown in Figure 5a, a distinctly different pattern was seen, although the histologic size grouping was the same (small groups of atrophied fibers). Many of the biopsies in this group showed a substantial number of angulated fibers (where the normal polygonal appearance is so altered as produce a much larger major axis in relation to a markedly reduced minor axis diameter). Generally, there was a suggestion of two separate peaks in the minor axis and three peaks in the major axis (as shown in Figure 5a). These peaks may represent the populations of abnormally small fibers, average-sized fibers and angulated fibers respectively. The variability within this pattern appears to be determined principally by the quantity of small fibers encountered.

4. Large groups of abnormally small fibers (Figs. 6 and 7)—62 patients. The characteristic histologic findings of "neurogenic atrophy" is the appearance of large groups of abnormally small fibers (Fig. 6). In Figure 7, muscle fibers were measured from two different muscles of the same patient. Although histologically they showed large segmental distribution of atrophy, they showed a considerable variability in size distribution pattern. Figure 7b shows a pattern more or less similar to that in Figure 5a. Two peaks in the minor axis diameter

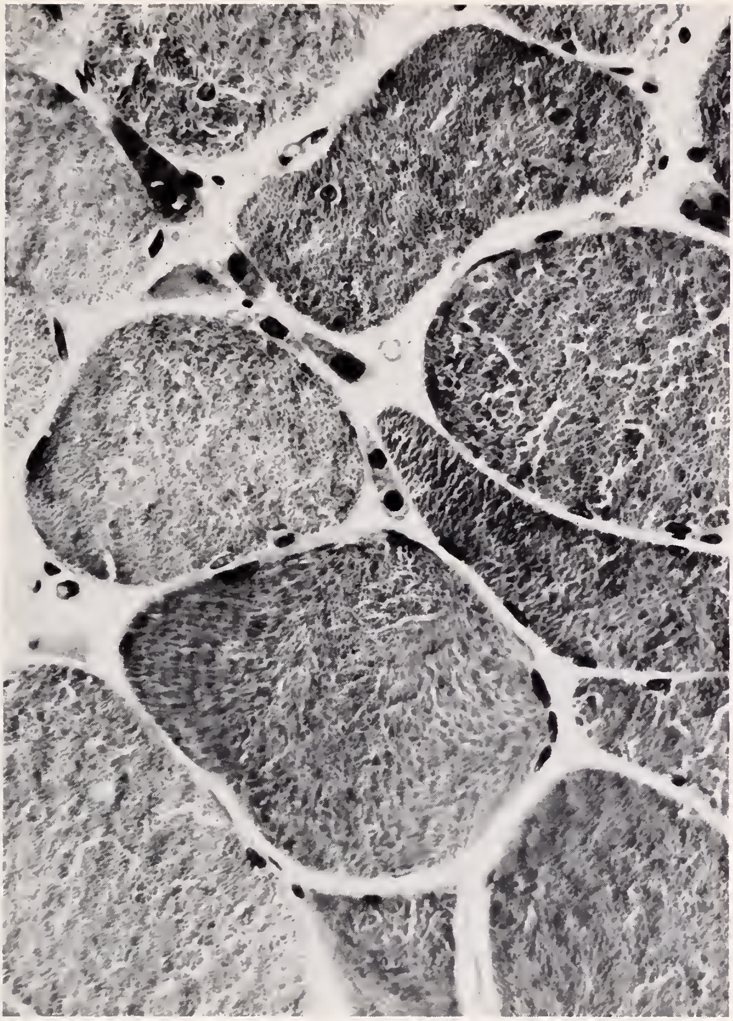

FIG. 4. Small groups of abnormally small muscle fibers, approximately × 650.

and three in the major axis diameter, are also evident here. The slightly different pattern displayed in Figure 7a is most likely due to the difference in the number of abnormally small fibers present.

The range fiber size was greater than the previous patterns, varying from 5 to 100 micra in the minor axis and from 8 to 130 micra in the major axis.

5. The occurrence of the various patterns of fiber size. In Table I, the incidence of each of the 3 patterns of atrophy was plotted against the clinical diagnostic impression. In each clinical group, approximately 20% (34 patients) had normal biopsies. However, in the overall neurogenic group, 36%, (62 patients) of cases showed large group atrophy, 26.7% (46 patients) showed small group atrophy and 17.4% (30 patients) showed random atrophy. There

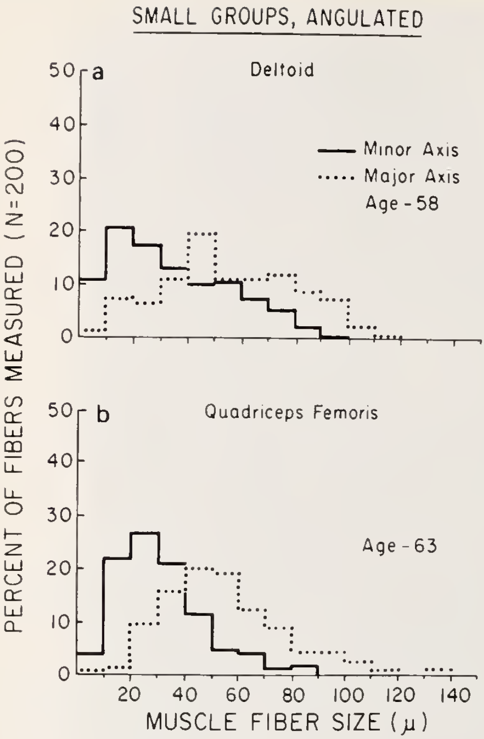

FIG. 5a, b. Small groups of abnormally small fibers—with angulated fibers. The variation in histographic pattern is shown (a–Deltoid; b–Quadriceps). There is a greater incidence of small fibers, and a wide range in fiber size.

was no significant difference in the occurrence of large groups between the myelopathies and the neuropathies.

In Table II, the entire neurogenic group of 172 patients was compared to a variety of myopathic disorders (including dystrophy, myositis, etc.—137 patients). The incidence of "large groups" in neurogenic disease was 36% (62 patients) compared to 15.1% (21 patients) in the myopathic group. The random distribution of abnormally small fibers was much less frequent in neurogenic (17.4%, 30 patients) than in myopathic disorders of muscle (49.6%, 68 patients), and the incidence of small group atrophy was the same in both groups.

Though large group atrophy was more common in the neurogenic group, they are certainly not limited to that group.

6. *Duration of neurologic illness and pattern of atrophy.* Table III shows the relationship of the duration of clinical illness to the patterns of atrophy. Cases of Werdnig-Hoffman disease were excluded from this comparison because of uncertainty concerning the duration of illness. There were 56 patients in which biopsy showed large group atrophy, 46 patients with small group atrophy and 30 patients with random atrophy. 26.8% (15 patients) of patients with large group atrophy had a clinical duration of illness of 6 months or less. One-half of the biopsies showing small group or random atrophy (23 and 15 patients respectively) occurred within the same period of illness. When a duration of

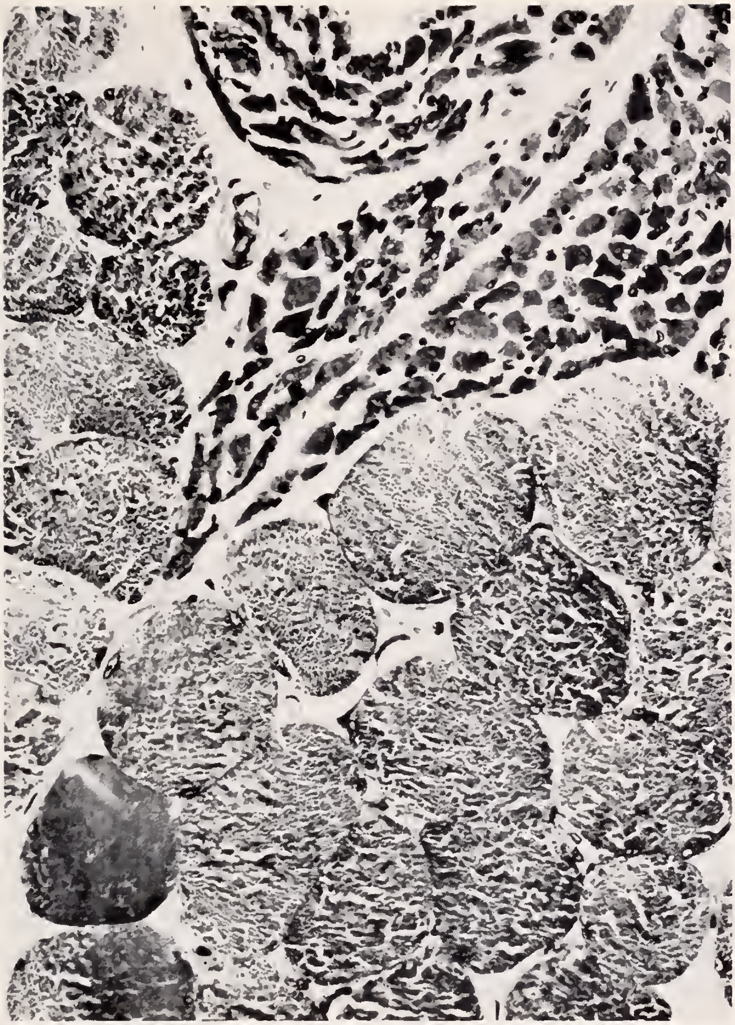

Fig. 6. Large groups of abnormally small muscle fibers approximately × 450.

illness of one year or more was considered, there was an incidence of 57.1% (32 patients) for "large groups", 45.6% (21 patients) for "small groups" and 30% (9 patients) for the random group. This suggests that with increasing duration of neurologic illness, the incidence of segmental atrophy increases.

Discussion

A histologic survey of neurogenic disease of muscle was performed as part of a five-year prospective study of muscle disease.

Patterns of abnormally small fibers divided into 3 groups—random, small and large groups of abnormally small fibers. The latter type is the classically accepted diagnostic feature of neurogenic disease of muscle, and its significance

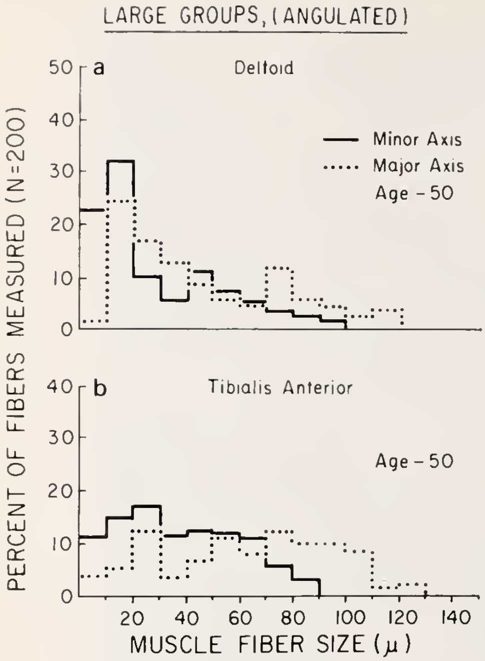

FIG. 7a, b. Large groups of abnormally small fibers—with angulated fibers. Distinct peaks are present in each axis—probably representing small, normal and angulated fibers (a–Deltoid, b–Tibialis Anterior).

TABLE I

Atrophy: Pattern of Distribution

	Cases	Large groups	Small groups	Random	Normal
Myelopathy	78	30 (38.5%)	24 (30.8%)	9 (11.5%)	15 (19.1%)
Neuropathy	85	29 (34.1%)	21 (24.7%)	18 (21.1%)	17 (20.0%)
Mixed	9	3 (33.3%)	1 (11.1%)	3 (33.3%)	2 (22.2%)
Total	172	62 (36.0%)	46 (26.7%)	30 (17.4%)	34 (19.8%)

TABLE II

Pattern of Atrophy—Neurogenic vs. Myopathic

	Cases	Large groups	Small groups	Random	Normal
Neurogenic	172	62 (36.0%)	46 (26.7%)	30 (17.4%)	34 (19.8%)
Myopathic	137	21 (15.3%)	36 (26.3%)	68 (49.6%)	19 (13.9%)

is confirmed in this study. The typical histologic appearance was that of large areas of adjacent abnormally small fibers, often involving an entire muscle bundle and corresponding to the motor unit.

When these patterns were plotted as histograms, relatively typical appearances were seen for each, but with a broad range of overlap. These patterns thus represent a wide spectrum in which one pattern blends into another

TABLE III

Duration of Neurologic Illness and Pattern of Atrophy

	Large groups	Small groups	Random
Cases	56*	46	30
1 Month	3 (5.4%)	2 (4.3%)	1 (3.3%)
1-6 Months	12 (21.4%)	21 (45.7%)	14 (46.7%)
7-12 Months	7 (12.5%)	2 (4.3%)	4 (13.3%)
1-2 Years	18 (32.1%)	11 (23.9%)	3 (10.0%)
>2 Years	14 (25.0%)	10 (21.7%)	6 (20.0%)
Unknown	2 (3.6%)	—	2 (6.7%)

* Excluding 6 cases of Werdnig-Hoffman

(ranging from the normal biopsy to the "large group" distribution), though each pattern appears somewhat distinctive.

When these patterns were plotted against the duration of illness, large group atrophy was encountered in slightly more than 25%, of patients with a duration of disease of 6 months or less, in contrast to the 57.1% incidence with a duration of one year or more. This may explain the difficulties encountered in biopsy interpretation of these patients. These data suggest that the time of biopsy may play an important role in the diagnosis of neurogenic disorders of muscle.

Conclusions

Three patterns of abnormally small fibers were seen in muscle biopsies from 172 patients with neurogenic disorders of muscle: large groups, small groups, and a random distribution. Large groups of abnormally small fibers were the most characteristic histologic finding in neurogenic disorders of muscle. This feature was observed more frequently in patients with a longer duration of illness. Characteristic histograms were shown for each "pattern of atrophy" and formed a broad spectrum in which some degree of overlap occurred.

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Simultaneous Tactile Perception in Patients with Conversion Sensory Deficits

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Various patterns of responses have been described in studies of the perception of simultaneous tactile stimuli (1). The pattern of correct localization of both stimuli within ten trials is characteristic for children over twelve years of age and normal adults (2–4). Persistent failure to identify one of the two stimuli (extinction) is found in younger children, mentally defective and senile adults, and adults with altered brain function marked by changes in sensorium (5–8). Mislocalization of percepts also occur in these subjects, the displacement along the long axis of the body being the most common (9). The errors in perception usually occur on both sides of the body, but in subjects with hemisensory or hemimotor syndromes, errors are more prominent over the involved side of the body or limited to this side. Errors occur more frequently over the hand and least over the face or genital regions, with other body parts between these areas in an "order of dominance."

During these investigations we studied a group of patients with conversion hysteria manifested by hemilateral or glove-stocking sensory deficits and sometimes hemilateral motor weakness (10). The perception of double simultaneous stimuli differed from patterns previously observed in patients with organic brain dysfunction.

Over fifty percent of patients with a hysterical hemisensory deficit to single stimuli showed correct perception on tests with double stimuli involving the affected side. This high percentage of correct responses to double stimuli in the presence of obvious deficits with single stimuli is in contrast to that found in patients with cerebral dysfunction. Such patients showing severe sensory deficits with single stimuli also invariably manifest errors with double stimuli. This type of dissociation, frequently found in the present study, appears characteristic for patients with conversion sensory changes.

Some patients showed a variety of perceptual errors which are not usually seen in patients with cerebral disease. The incidence of errors varied with the

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pattern of stimulation. Invariable extinction of one of the two stimuli occurred when the stimuli were applied homolaterally to the affected body areas while contralateral stimuli (symmetrical or nonsymmetrical) were always perceived correctly. Despite such frequent extinction with homolateral stimuli, displacement of stimuli did not occur. There was also a fluctuating order of dominance in which body areas other than the face or genital region were perceived most frequently.

In contrast, patients with unilateral brain disease do not show this type of dissociation. If there is frequent extinction on homolateral testing over the affected side, errors also occur in tests with contralateral stimuli (symmetrical or nonsymmetrical). In addition, there is an incidence of displacement of stimuli if extinction is frequent and the face and genital areas are most dominant.

A small group of patients with severe sensory loss showed what may be called "apparent extinction." Intermittently during the course of testing, one of the two stimuli was not felt over some parts of the affected side of the body. However, at these times single stimuli to these areas interspersed with simultaneous stimuli were also not perceived. This "apparent extinction" was a reflection of the fluctuating perception of all stimuli, double as well as single. During other periods of testing, when single stimuli were perceived correctly, extinction responses disappeared.

One aspect of the responses of patients with conversion phenomena to tests of double simultaneous stimuli is shared with other forms of hysterical dysfunction. They frequently exhibit a rigid all-or-none quality. Motor weakness occurs at all joints of the extremity and sensation is equally defective over large, well-demarcated areas. The usual gradients of dysfunction seen in organic disease are missing. Similarly, errors with double simultaneous stimuli occur in a rigid manner over all parts of the body and are manifested on nearly all tests with one method of testing and yet are entirely absent with another method. Despite a high incidence of extinction, there are no instances of ipsilateral displacement as a manifestation of a gradient of dysfunction.

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Synaptic Linkage of the Vestibulo-Ocular Reflex Pathways in the Rabbit

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The vestibulo-ocular reflex pathways have been investigated by the observation of nystagmus and evoked eye movements during stimulation of the labyrinth, vestibular nuclei, and cerebellum (1–4), by observation of the effect of small lesions placed within the vestibular nuclei upon behaviorly evoked eye movements (5), and by anatomic techniques employing the staining of degenerating axons (6–8). These vestibulo-ocular pathways have now been investigated with electrophysiologic techniques (9–15). This communication will review these recent investigations.

It was shown (9–11, 14, 15) that by electric stimulation of the eighth nerve, both inhibitory and excitatory postsynaptic potentials (IPSPs and EPSPs) are produced in rabbits' IIIrd, IVth, and VIth nuclei. The IPSPs in the IIIrd and IVth nuclei are mediated by a pathway arising exclusively in the superior vestibular nucleus (SV) and traversing the fasciculus longitudinalis medialis (FLM). The IPSPs in the VIth nucleus are mediated by the medial vestibular nucleus (MV) (15). There is evidence (12, 13, 15) indicating that the inhibitory transmitter is γ-aminobutyric acid (GABA) or a related substance. EPSPs in the IIIrd nucleus were shown (9–11) to be mediated via two separate pathways, one arising in the rostral half of the MV and traversing the FLM, and the other arising in the lateral nucleus of the cerebellum (LN), and the Y-group of the vestibular nuclei (YG) and traversing the brachium conjunctivum (BC) to the IIIrd nucleus. EPSPs in the IVth and VIth nucleus were shown to arise in the MV (10, 14, 15). Further, the convergence of these inhibitory and excitatory pathways onto each subgroup of motoneurons within the IIIrd, IVth and VIth nuclei innervating different extraocular muscles was established (14, 15).

Methods

All experiments were performed on adult rabbits (3–5 Kg) anesthetized with pentabarbitone sodium and paralyzed with gallamine triethiodide. Recording sites in the IIIrd and IVth nuclei were reached with glass microelectrodes by penetration of the superior colliculus after removal of the ipsilateral occipital cortex. The VIth nucleus was reached by penetration of the cerebellum. Gross identification of the IIIrd, IVth, and VIth nuclei was made by observing the antidromic field potentials evoked by stimulation of the IIIrd nerve (see Fig. 1 labeled ANTI), IVth or VIth nerves with stereotaxically-implanted electrodes.

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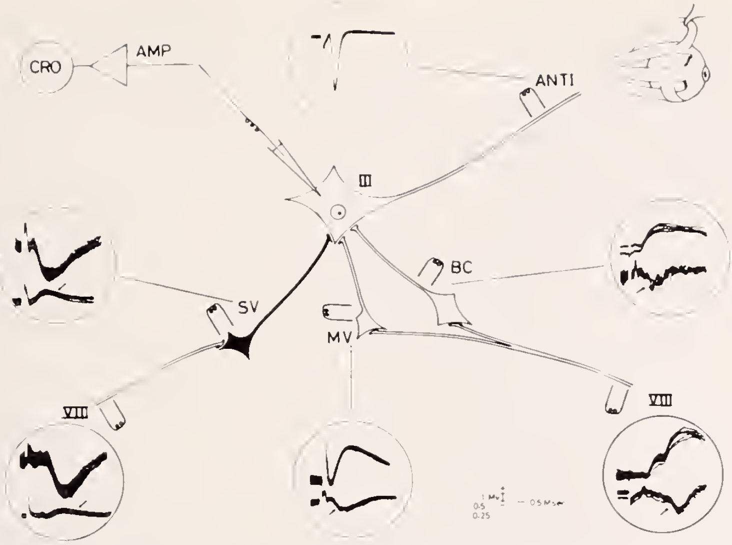

Fig. 1. Electrical activities in oculomotor nuclei. Potentials recorded from the IIIrd nucleus are encircled and related to the sites where stimulation was employed. ANTI = extracellularly recorded antidromic field potential evoked by stimulation of IIIrd nerve. In all other records, upper traces are intracellular records and lower traces are corresponding extracellular potentials. VIII either VIIIth nerve; III = oculomotor neurone; AMP = amplifier; CRO = cathode ray oscilloscope. In this and in Fig. 2, positivity is represented by upward deflection. All records were formed by superimposing about 10 traces repeated at a rate of 2–5/sec. Arrows point to the negativity and positivity in the lower traces of the appropriate records. Calibration is 0.5 msec for all traces and 1 mV for lower traces in BC. Note the latencies of onset of IPSP, EPSP, and field potentials are shorter after stimulation of SV, MV, or BC, than after stimulation of VIII.

If subgroups of IIIrd nucleus motoneurons innervating different eye muscles were to be studied, identification of each of these subgroups was accomplished through stimulation of the nerves innervating each individual muscle. Since the motor nerves are relatively short, care was taken to avoid current spread between adjacent nerve branches. The intensity of stimulation applied to each nerve branch was adjusted to produce a just maximal activation by observing the antidromic field potentials and it was confirmed that at this intensity of stimulation, there was no interference between the fields evoked by stimulation of the different branches. With nerve cells having a stellate configuration, such as oculomotor neurons, excitation of the somatic region will be accomplished by the development of extracellular negative potentials which have their maximum in the center of the field in proximity to the cell body and radially decrease in all directions away from the center (closed field of Lorente de Nó (16)). Therefore, the site of maximum antidromic field potentials evoked by a particular branch nerve stimulation should be coincident with the site of the maximum density of cell bodies of the corresponding subgroup of motoneurons. Orthodromic activation of these neurons was accomplished by stimulating both VIIIth nerves in the periphery and by direct stimulation of the medulla. The medullary stimulating technique was different for each of the two distinct types of experiments performed. In one series of experiments, bipolar stimulating electrodes were placed within various portions of the brain stem and cerebellum and the effects of stimulation were surveyed with intracellular recording in the IIIrd, IVth, and VIth nuclei. The positions of these stimulating electrodes were adjusted by observing the extracellular field potentials produced in IIIrd, IVth, or VIth nuclei by stimulation, for these reflected the intracellular events very well, as will be shown below. In the second series of experiments, systematic tracking of the medulla and cerebellum was performed with a monopolar electrode while a microelectrode was placed within various portions of the IIIrd, IVth, or VIth nuclei to record field potentials. With stimulating current limited to 1 mA with bipolar and 50–100 μA with monopolar stimulation, the effects of stimulation could be shown (9) to be localized to the area immediately surrounding the electrode tip. Results of the second series, or tracking experiments, were displayed by plotting the effective sites of stimulation both on histologic sections and on a standardized dorsal view of the medulla. Accurate localization of these sites was obtained by referring the readings on the micromanipulator scale for the stimulating electrode to lesions placed within the tissues during the experiment. Residual traces of electrode tracks could also be seen histologically.

Results

Results of intracellular recording within the IIIrd nucleus are as follows. Stimulation of the VIIIth nerve often evoked IPSPs in oculomotor neurons (Fig. 1). Stimulation of a localized rostral portion of the medulla could also evoke IPSPs similar in size and configuration but with significantly shorter latencies (Fig. 1), short enough in fact to indicate that these medullary IPSPs are evoked monosynaptically. Since the latency difference between the medullary- and VIIIth-nerve-evoked IPSPs is of the order of one nuclear delay, the medullary-evoked IPSPs are presumed to be produced by direct stimulation of the inhibitory secondary vestibular neurons. If the VIIIth-nerve stimulation evoked disynaptic IPSPs, ipsilateral medullary stimulation invariably evoked monosynaptic IPSPs of similar or larger amplitude. Eighth-nerve-evoked IPSPs are always completely occluded by medullary-evoked IPSPs when produced simultaneously. These results indicate that the rostral portion of the vestibular nuclear complex is the exclusive site relaying the inhibitory pathway from the ipsilateral VIIIth nerve to the IIIrd nucleus.

By a similar line of reasoning and investigation (9), the excitatory or EPSP pathway to IIIrd nucleus was shown to have two medullary relay sites, one, the middle portion of the vestibular nuclear complex and the FLM, and second, certain cell groups of origin of BC; both of these sites relay excitation from the ipsilateral VIIIth nerve to IIIrd nucleus.

To further localize these pathways, a more systematic approach was used.

Although intracellular recording provides the most direct measure of certain synaptic parameters, its drawback is that it is difficult to maintain stable recording for a long time. Therefore, the extremely stable extracellular field potentials were utilized during the systematic tracking series of experiments. Because field potentials are an indirect reflection of the average membrane potential changes of a group of cells, they must be cautiously interpreted. However, by combining both intra- and extracellular recording techniques, their mutually exclusive shortcomings could be overcome. In this regard, it was noted that in the region of cells exhibiting EPSPs after VIIIth nerve, medullary, or BC stimulation, the extracellular fields are composed of spikey prepotentials representing incoming volleys, and slow negativity representing current flowing into the activated subsynaptic membrane plus the action currents of activated cells (Fig. 1). In contrast, in the region of cells exhibiting IPSPs after VIIIth nerve or medullary stimulation, a spikey prepotential plus a slow positivity is recorded, representing respectively incoming volleys and outward current flowing across the inhibitory subsynaptic membrane (Fig. 1).

Monosynaptic initiation of EPSPs and IPSPs in oculomotor neurons can thus be recognized by the appearance of negative or positive field potentials with a short latency of approximately 1 msec. These interpretations of field potential were also found to apply to the IVth nucleus. Although the configuration of the spikey prepotentials recorded in the VIth nucleus after VIIIth nerve or medullary stimulation was not as distinct as in the IIIrd and IVth nuclei, similar interpretations of the positive and negative field potentials were validated.

Analysis of the results of many tracking experiments led to the postulates (9, 10, 14) that the inhibitory secondary vestibular neurons for the IIIrd and IVth nuclei are contained exclusively within the SV, while the excitatory secondary vestibular neurons are contained within the rostral two-thirds of the MV, as well as in the BC related structures, the Y-group and the LN of the cerebellum. Figure 1 diagrams these connections and the corresponding intra- and extracellular potentials recorded in the IIIrd nucleus after VIIIth nerve, MV, BC, and SV stimulation. The inhibitory secondary vestibular neurons for the VIth nucleus are contained within rostral MV and the excitatory secondary vestibular neurons also lie within the rostral MV.

The convergence of these inhibitory and excitatory pathways onto each subgroup of motoneurons in IIIrd, IVth and VIth nuclei was investigated. To do this, the recording electrode was placed within each subgroup of motoneurons by observing their antidromic field potentials evoked by stimulation of the individual extraocular muscle branch nerves. The antidromic potentials overlapped somewhat between different subgroups, presumably due to overlapping dendritic trees; however, in accordance with the results obtained by anatomical techniques (17), the cells were arranged in 1–2 mm long antero-posteriorally oriented groups with some layering of the groups along the vertical axis. As expected (17), the cell bodies of superior rectus (SR), and superior oblique (SO) are located contralateral to the side stimulated, while those of medial rectus (MR), inferior rectus (IR), inferior oblique (IO) and lateral rectus (LR) are ipsilateral. In addition, the cell bodies of SR lie predominantly postero-medially, those of IR mainly antero-laterally, while those of MR lie ventro-medially.

Figure 2 demonstrates that with the recording electrode located within the left SO nucleus, stimulation of the right VIIIth nerve (Fig. 2, R VIII) gave rise to negative potentials indicating disynaptic excitation of SO neurons, while

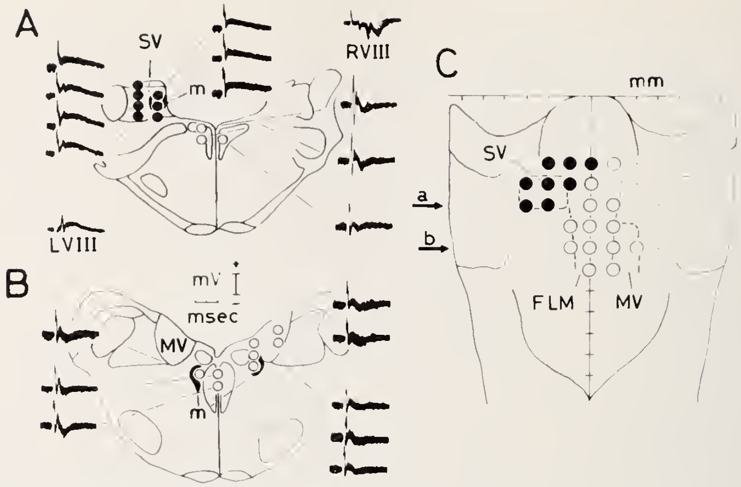

Fig. 2. Map of the spots effective in producing inhibition and excitation of the cells of the left superior oblique nucleus. a and b: Cross sections of the medulla and cerebellum drawn from histological sections of the brain. c: Standardized dorsal view of the medulla. Dotted lines in c indicate the borders of the structures labeled as determined by projecting their actual borders seen on cross sections, onto the floor plan of the medulla. Section a was taken at the level marked by arrow (a) at the right side of c, and section b from the site of arrow (b). Representative potentials have been attached to the stimulated sites from which they were evoked. ○ excitation related to the MV-FLM pathway, and ● inhibition related to SV. L VIII is the potential produced by stimulating the left VIIIth nerve and R VIII by stimulation of the right VIIIth nerve. Note that potentials evoked by medulla stimulation have shorter latencies than those of L VIII or R VIII.

stimulation of the left VIIIth nerve (Fig. 2, L VIII) gave rise to positive potentials indicating disynaptic inhibition. The results of a systematic tracking experiment to ascertain the pathways through which the VIIIth nerve impulses produced these negative and positive potentials are illustrated. In the cross sections of the medulla a and b made at the two levels indicated by the arrows (a) and (b) in Fig. 2c, representative potentials recorded in the SO nucleus all attached to the sites from which they were evoked. As mapped in these cross sections as well as on the floor plan in Fig. 2c, short latency negativity (○) was found to arise from the rostral part of MV and was traced rostrally along the FLM to the IIIrd nucleus. The site of excitation was contralateral to the recording site. Positive potentials of short latency (●) arose exclusively from SV ipsilateral to the recording site. Intracellular recording of IPSPs and EPSPs from 17 SO cells was in full agreement with the results of these tracking experiments.

Similar tracking experiments were repeated for each division of the IIIrd, IVth and VIth nuclei, and the results were confirmed with intracellular recording from 17 to 66 cells of each division. As the summary in Fig. 3 indicates, inhibition of IR arises from ipsilateral SV and excitation of IR from contralateral MV-FLM and LN-BC pathways; inhibition of IO arises from the ipsilateral SV and excitation of IO from contralateral MV-FLM and the Y-group-BC pathways; inhibition of MR was bilateral from both the SV and excitation

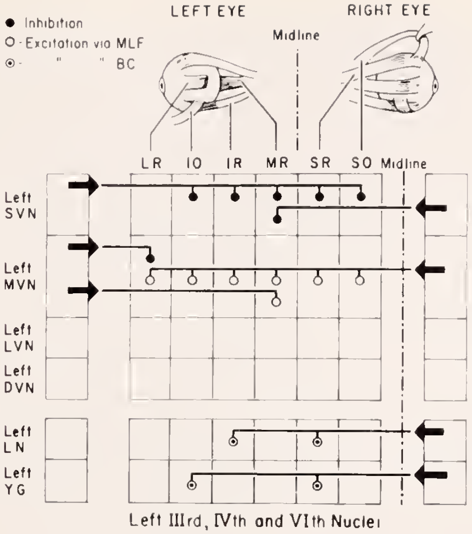

Fig. 3. Vestibulo-ocular connections to the left IIIrd, IVth and VIth nuclei in the rabbit. Central block of six columns represents the left IIIrd, IVth and VIth nuclei, the long blocks on the right and left of the main block represent relay nuclei of the vestibulo-ocular reflex arcs on both sides. Note that the IO, IR and MR divisions of the left IIIrd nucleus as well as the LR are ipsilateral to the left eye while the SR and SO innervate the right eye. ● Inhibitory connections from the SV; ○ excitatory connections from the MV; ⊙ excitatory connections from the Y-group or LN. LSV, RSV = left and right superior vestibular nuclei, respectively. LMV, RMV = left and right medial vestibular nuclei, respectively. LLN, RLN = left and right lateral nuclei of the cerebellum, respectively. LYG, RYG = left and right Y-groups of the vestibular nuclei, respectively.

of MR bilateral from both MV; and that SO (IVth nucleus) is inhibited by the ipsilateral SV and excited by the contralateral MV. In other words, SV transmits inhibition to the ipsilateral cell bodies of the entire IIIrd and IVth nuclei, while contralateral MV transmits excitation to these same cells. In this convergence of SV and MV that is principally unilateral, the MR division of the IIIrd nucleus is the exception, receiving bilateral inhibition from both SV and bilateral excitation from both MV. BC mediated excitation is crossed, arising from the Y-group for IO, from both the Y-group and LN for SR, and from the LN alone for IR.

Concerning the vestibulo-ocular pathways to the VIth nucleus the inhibitory relay cells could be effectively stimulated within the rostral half of the ipsilateral MV while the excitatory relay cells could be stimulated within the rostral half of the contralateral MV (15). In view of the location of the inhibitory relay neurons for the vestibulo-ocular reflexes to the IIIrd and IVth nuclei it is suprising that stimulation of the SV did not produce short latency effects in abducens motoneurons. However, close similarity was demonstrated between the vestibular inhibition for the VIth nucleus and that for the IIIrd nucleus as noted by the following two points. First, pharmacological investigation (15) indicated that the vestibular inhibition in the VIth nucleus (15) is mediated by GABA or a related substance just as in the IIIrd nucleus (13). Second, the inhibitory pathways to both the IIIrd (18) and VIth (15) nuclei are under inhibitory control of the flocculus. Thus, the inhibitory relay cells for both the IIIrd and VIth nuclei are functionally, if not anatomically, identical.

Of interest is the involvement of the Y-group and the LN in the excitatory vestibular ocular reflex arc. The Y-group has long been included among the vestibular nuclei (19, 20) by virtue of its primary vestibular fiber input, recently shown (21) to be derived from the saccule. Stimulation of the saccule can produce patterned eye movements (22), while destruction of this organ is associated with a decrease in ocular counter rolling (23). Counter rolling is also known to have a dynamic and a static component. It is likely that the saccular contribution to eye movements, and perhaps the dynamic component of counter rolling in certain head positions, is mediated at least in part, by the Y-group-BC pathway. Primary vestibular input to the LN has been identified anatomically (24) but its peripheral source has not yet been specified. The LN has been shown (6) to project to the IIIrd nucleus through the BC, and to influence ongoing activity in the IIIrd nucleus neurons (25).

The results of the present experiment concerning the projections of the SV and the MV are in partial agreement with the results of degeneration studies. In the monkey (7) it was suggested that the projection of the SV was exclusively ispilateral and limited to certain cell groups, while the projections of the MV were found to be bilateral to all subgroups of motoneurons. In the cat (8, 26) it was suggested that the projection of SV was bilateral to IIIrd and IVth nuclei and ipsilateral to VIth nucleus, while MV projected contralaterally to all subdivisions but only to ipsilateral IR and MR divisions of IIIrd nucleus and to VIth nuclei bilaterally. In the rabbit (27) it is assumed that SV projects bilaterally also. In this context SV and the BC are located very close together although they have completely different projection patterns to IIIrd nucleus. This could complicate the interpretation of degeneration experiments. Species difference may also be a factor.

Even though there is still a gap between the electrophysiologic and behavioral investigations, there are certain points of agreement. Simultaneous electrical stimulation of the three semicircular canals on one side should cause contraction of the ipsilateral SR, SO, and MR and contralateral IR, IO, and LR extraocular muscles, plus their secondary synergists the ipsilateral IO and IR and the contralateral IR and SO muscles (24, 28). Because of the short latency to onset of eye muscle EMG after stimulation of the vestibular organ, it was suggested that these muscles were activated by the disynaptic pathway through the vestibular nuclei (2). The present results partially confirm that postulate. The necessary disynaptic pathways to all of the primarily-excited muscles were shown to exist, however, for some of the secondarily-activated or synergist muscles, i.e., the ipsilateral IO and IR and the contralateral SO; the necessary connections for disynaptic activation were not found.

This discrepancy might be due to the fact that (1) simultaneous stimulation of the whole VIIIth nerve in the present experiment suppressed conflicting inputs; (however, direct stimulation of the medulla should have eliminated this possibility); or (2) the anesthesia used in the present experiment might have suppressed polysynaptic pathways which in general are dominant in the alert animal.

It is expected that this conflict will soon be resolved, and that continuing research will be productive in further elucidating that physiology of the vestibuloocular system.

Acknowledgment

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Coupling between a Symmetric and an Asymmetric Neural System*

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Almost half a century ago, Pavlov reported that conditioned reflexes which had been elaborated on the skin surface of one-half of the body were obtainable to exactly the same degree from stimulation of corresponding symmetrical points of the other half of the body, even though the latter had never been tested before (1). It was, furthermore, impossible to obtain the slightest differentiation between two symmetrical points on opposite halves of an animal by use of the conditioned reflex technique; yet this was easily accomplished with asymmetrical, or nonhomologous points. In an experiment prescient of contemporary research on hemispheric specialization, the corpus callosum was then sectioned and the whole phenomenon was abolished.

When Dr. Morris B. Bender introduced the method of double simultaneous stimulation as part of the routine neurologic examination (2), the stage was set for a renewed interest in bilateral perceptual symmetry. Whereas the "face-hand test," a bilaterally *asymmetric* stimulus pattern, was a sensitive indicator of transient organic mental syndrome (3), bilaterally *symmetric* stimulus configurations proved to be remarkably stable (4). This phenomenon was reminiscent of Pavlov's observations and reminds us once more that a firm expectation of bilateral symmetry is a useful background against which to assess normal or pathologic asymmetries that may be of interest.

This same expectation of bilateral symmetry in normal hearing and oculomotor function is the background against which hemispheric specialization is currently studied. A recurrent notion in contemporary research on language mechanisms is that handedness and speech dominance may be independent (5–7). The possibility of such independence has been obscured by: a) the statistical predominance of left laterality for both functions; b) the fact that dominance is not an "all-or-none" phenomenon, but is rather a frequency function of a random variable; and c) the fact that the two major methods for inferring speech dominance, i.e., the intracarotid sodium amytal test (6) and the dichotic listening test (8), have not yet been compared in a group of normal subjects (9).

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One possible interpretation of the still controversial literature on the subject is that dominance for expressive language functions in the frontal lobe may indeed be correlated with handedness, but that *both* of these anterior functions may be independent of receptive language dominance in the temporal lobe. If this hypothesis is correct, there exist at least two linguistic asymmetries which may or may not be concordant in a given brain. Thus there would be four ideal types; left expressive-left receptive and right expressive-right receptive (concordant); left expressive-right receptive and right expressive-left receptive (discordant). A strategy to unravel the phenomenon would be to select subjects who exemplify the four ideal types of asymmetry and to test them on linguistic tasks (speaking and listening), the dependent variable being a neural system which operates symmetrically under non-linguistic control conditions. An ideal candidate for the latter is the oculomotor system (EOG) since the close anatomic association between Broca's area and the frontal eye field externalizes the expressive laterality as conjugate deviation of the eyes away from the dominant motor speech area (10, 11).

Within the above paradigm our laboratory has studied six normal right-handers (12). A uniform left lateralization of Broca's area was inferred from the tested manual preference (13). A left lateralization of Wernicke's area was inferred for three cases on the basis of the dichotic listening test (concordant type), and a right lateralization was inferred for the remaining three cases (discordant type). The initial EOG bias on listening to consonant-vowel syllables is of special relevance to this hypothesis. Stimuli were delivered binaurally, to each ear at 20 dB above its respective speech reception threshold. The EOG was averaged for 100 syllables, triggering on the peak amplitude of the vowel. The two groups were sharply contrasted by the EOG bias in the 0.2 sec poststimulus. Whereas the concordant types looked to the right, the discordant types looked first to the left and then to the right. We found this initial "eyes left" movement, beginning before the termination of the stimulus, in each of the discordant and in none of the concordant subjects. Assuming the probability of this biphasic shift is one-half in the population as sampled, it is significantly related to receptive speech dominance at the 0.02 level. One behavioral consequence of the two different organizations of speech dominance in these right-handers has thus been demonstrated.

Some neurophysiologic speculation may order these findings. The model assumes that Broca's and Wernicke's areas, along with important subcortical connections which are here omitted for the sake of brevity (14, 15), function as a reverberating circuit during speech related tasks. The bidirectional transmission (16) in our concordant type of dominance is classically handled by the left arcuate fasciculus. The straightforward, stimulus-locked, initial deflection of the eyes to the right in this group is predicted by the model. However, in our discordant type the connectivity between right Wernicke's and left Broca's areas is hypothesized to possess two options: a) via the right arcuate fasciculus, the region homotopic to Broca's area, and the corpus callosum anteriorly; or b) via the corpus callosum posteriorly, the region homotopic to Wernicke's area, and the left arcuate fasciculus. The stimulus-locked, initial left-right deflection of the eyes in this group suggests that the first option is being utilized inasmuch as the second option would not activate the right frontal eye field. A method is therefore suggested whereby eye movement bias might be utilized to label the route by which the anterior and posterior speech areas "find each other." If confirmed in an analogous group of left-handers, i.e., right expressive-right receptive (concordant) and right expressive-left receptive (discordant), the model could be of theoretical significance in cases of "crossed aphasia" (16) and conduction aphasia (17).

In summary, we find a coupling of asymmetric and symmetric neural systems such that induced bias in the latter furnishes clues to the transmission pathways of the former. In this and other unexpected ways (18), the methods and interests of the mentor have elucidated the research questions of the student.

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The Routine EEG: Its Use and Abuse

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Assuming the validity of the principle that all laboratory tests must have a definite purpose in the management of the patient, there should be no such thing as a "routine test." Some tests are so widely applicable, however, that almost all patients require them. Blood counts and urinalyses, for example, serve a valuable function as survey tests, providing leads to conditions which might not be otherwise suspected. Serologic tests for syphilis are an example of true screening tests—highly specific techniques which, if negative, "rule out" a condition, even though, if positive, they do not establish a diagnosis.

In neurology certain tests are also so common as to be ordered on a fairly "routine" basis. Literally millions of skull and spine x-rays are performed, often without any concrete notion of the potential value of the study. Similarly, EEG's are ordered in the hundreds of thousands yearly, often by internists or even neurologists, who are acting "for the sake of completeness" or "routinely."

How many of these tests are genuinely indicated? In how many cases does the clinical situation warrant the effort and cost involved? Prudence requires us first of all to recognize that these questions exist, and then to attempt to answer them.

The first principle to be established is that the EEG cannot be a "screening test" in the true sense. The EEG is merely a recording of a portion of the electrical activity of the brain, and, as such, can neither "rule out" nor diagnose any disease process. There are many conditions, however, in which the EEG can contribute significantly to diagnosis and therapy. These must be recognized. Conversely, there are circumstances in which the EEG, although commonly done, is at best useless, at worst misleading.

Most physicians think of the EEG primarily in connection with the epilepsies—an association which is fully justified. Where a clinical picture of convulsive phenomena exists, whether the paroxysmal manifestations be motor, sensory, or in the sphere of psychic states or general behavior, the EEG is absolutely essential. It must be emphasized, however, that unless an actual seizure is observed clinically and simultaneously recorded electrically, the EEG cannot confirm a diagnosis. Contrary to statements often expressed, there is no such thing as a diagnostic EEG in epilepsy. The diagnosis must always be made on clinical grounds. Even the most "typical" of all epileptic EEG patterns, the three per second spike and wave paroxysm ("petit mal" pattern) may be seen in persons who have only generalized convulsions or even no clinical seizures, as well as those who have petit mal absence attacks.

As a survey test in actual or suggested epileptic syndromes, the EEG is

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valuable primarily as an indicator of the underlying pathophysiologic mechanism. The finding of an intense slow-wave focus, or a zone of suppressed activity, would suggest that further studies be done to elucidate the possible existence of an arteriovenous malformation, benign tumor, or porencephalic cyst. The finding of bisynchronous spike and wave patterns would, in most cases, obviate the necessity of radiographic contrast procedures and aid in determining the medication to be used. Localized random spiking, whether fixed in position or shifting, whether unilateral or bilateral, would, according to the age of the patient and the severity of the clinical syndrome, give leads as to the medical or even surgical management of the disorder. Even a normal EEG may be highly significant, for example, in a patient who appears to be having absence attacks of the typical "petit mal" form. A normal EEG in such a case is very strong evidence that the seizures are actually brief automatisms (psychomotor seizures), due to an epileptogenic lesion involving the mesial temporal cortex or rhinencephalic structures.

The preceding remarks are not intended to provide a comprehensive review of the use of the EEG in epilepsy. They are meant to indicate, by a few examples, the complexity of the variables involved, and the consequent need for sophistication in approaching the clinical problems of epilepsy with this laboratory tool (1, 2).

One illustration of this principle may be found in relation to the case of the EEG in infants who have had febrile convulsions. This practice is "routine" in many hospitals and clinics, in spite of the effort and expense involved in the performance of EEG's on patients of this age. In a recent survey of one hundred consecutive EEG's done in febrile convulsion patients at The Mount Sinai Hospital, only three abnormal records were found, and of these only one showed an abnormality of "epileptic" type (3). A well-controlled longitudinal study of children who had experienced "simple febrile convulsions" (single seizure, duration less than 5 minutes, no prolonged cyanosis, no focal neurologic sequelae) showed that the EEG was rarely abnormal, and of no prognostic value in predicting the later development of convulsive disorder (4). In view of this evidence, it seems that EEG's in this group of patients, unless done as part of a prospective research project, are a waste of effort.

Conversely, there are many instances where investigation is terminated in seizure patients because of a report of a "normal EEG" when only a relatively brief resting record has been performed. If the clinical problem is a significant one, however, truly adequate EEG studies require prolonged recording in the resting state, special electrode placements, sleep records, sleep deprivation studies, and possibly chemical activation.

An example of relatively common and yet essentially valueless use of the EEG is in unselected headache patients. Acting, one presumes, on the a priori assumption that such tests are of diagnostic or prognostic value, most "headache authorities" tend to recommend an extensive battery of screening tests, including the EEG (5). We recently tested this assumption by reviewing the EEGs of one hundred consecutive patients referred from The Mount Sinai

Hospital Headache Clinic. All patients in this group of ambulatory chronic headache sufferers, ranging in age from 14 to 72, had normal EEG's. This finding is somewhat paradoxical, especially in the older patients, in whom a certain percentage of random abnormalities might be expected (6), in that it suggests a statistical bias in *favor* of normality in the EEG of headache patients. In any event, the most obvious conclusion is that doing EEG's in "headache patients" is of no greater value than doing them on people chosen at random among the passengers on a municipal bus. Similarly meaningless and baselessly reassuring "normal" EEG's can be obtained in groups of people suffering from ill-defined syndromes of giddiness, lightheadedness, and faintness.

The EEG can be of great value as a survey procedure in patients who present with transient or subtle focal motor or sensory signs. For example, if a patient in the age group wherein vascular insufficiency phenomena are common has a transient hemiparesis, a contralateral focus of continuous high voltage slow activity would indicate the probable evidence of an active expanding or destructive lesion and the consequent need for aggressive management. A normal EEG, while not ruling out neoplasm, would be evidence suggesting a more conservative "watchful" approach to the case. Another very valuable, although unusual result in such cases is the "paradoxical" (apparently wrong-sided) focus. When a clear-cut unilateral focal abnormality in the EEG exists on the side opposite that predicted by clinical phenomena, it means that the clinical lateralization is erroneous, or that the patient has more than one lesion (7). The most likely circumstances under which this can occur is the presence of metastastatic carcinoma or subdural hematoma.

It must be emphasized that the proper use of the EEG, even as a survey procedure, often requires that serial studies be performed. The EEG is of particular value as a monitor of fluctuating or evolving states of cerebral dysfunction. This quality is exemplified in cases of minor head injury. In general the EEG is unlikely to be revealing in cases of cerebral concussion or minor closed head injury without loss of consciousness. In middle-aged or elderly patients, however, serial EEG's after minor head injury can be the most sensitive indicator of the presence of subdural hematoma (8).

Initial survey and serial EEG's can also be of great value in the differential diagnosis of coma. In many cases obvious causes such as trauma, subarachnoid hemorrhage, and diabetic acidosis are lacking. The EEG may sometimes help by showing the fairly characteristic, diffuse, frontally accentuated, highly synchronized stereotyped polyphasic sharp complexes of metabolic (particularly hepatic) encephalopathy. It may rarely show the continuous epileptic discharges of pseudo-coma due to "psychomotor" or "petit mal" status epilepticus (9). The EEG may also show the relatively normal-looking low-voltage mixed frequency activity which in the presence of coma indicates the existence of either a severe lower brain-stem transverse lesion or acute barbiturate intoxication (10).

The literature on the subject of survey EEG's in childhood learning problems and behavior disorders is a hopeless tangle of conflicting results and interpretations. Much of the confusion derives from the lack of well-established, universally recognized clinical criteria in these conditions. Equally important is the similar lack of established and accepted criteria for "normality" in the EEG's of children (11). These defects are compounded by the almost total absence of rater-reliability checks and adequate statistical sampling controls in these studies. Obviously, under proper circumstances, valuable research in this area can be done, and is being done. As a method in clinical management of individual cases, however, the survey EEG is of very limited value. Very rarely, a child who shows only defects in learning and attention may actually be shown with the aid of EEG to be having almost constant "sub-clinical" petit mal seizures. Even more rarely focal slowing or suppression may indicate the presence of an unsuspected cystic lesion or angioma of one cerebral hemisphere.

As part of an ongoing clinical research project, EEG's were performed on 167 pediatric psychiatry inpatients at The Mount Sinai Hospital between 1963 and 1967. The average age was 9 years, and the clinical diagnosis in most cases was juvenile schizophrenia or behavior disorder. 124 patients were tested both awake and asleep. Only 15 patients had abnormal EEG's, and of these 11 had gross clinical evidence of "organic brain dysfunction" (convulsive seizures, hemimotor syndromes, retardation). The paucity of paroxysmal or focal EEG abnormalities in these severe behavior disorder cases is striking and illustrates the relative unproductiveness of the survey EEG in such cases.

In the practice of general psychiatry (especially in hospitals, but even in ambulatory patients), any clinical evidence of organic brain dysfunction is ample justification for the performance of an EEG. In the younger age group, the EEG may help to indicate the presence of drug intoxication or withdrawal syndromes. In older patients with organic mental syndromes, the EEG can be valuable evidence of an unsuspected focal expanding lesion, or of some progressive diffuse degenerative disease. Whether clinically "ill" or merely decompensating to the point of requiring change from individual life to custodial care of some sort, any organic mental patient merits careful clinical appraisal and basic laboratory survey studies, including an EEG.

Conclusions

This paper has discussed the value of the EEG as a survey procedure in conditions such as epilepsy, coma, and organic mental syndrome. It has shown its misapplication in conditions such as tension headaches and simple febrile seizures. The results cited and the concepts elucidated lend strength to the principle that a specific purpose should be in mind whenever the EEG is ordered. In order that the test may be of value in the clinical situations involved, the physician must have a clear understanding of the potential significance of the results, whether "positive" or "negative."

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Oculomotor Palsy with Pupillary Sparing in Subdural Hematoma:

Two Cases with Documented Tentorial Herniation

JAMES R. KEANE, M.D.*

Introduction

The appearance of a dilated fixed pupil in association with expanding cerebral masses has been observed for over a century. An ipsilateral dilated pupil is by far the commonest eye sign of subdural hematoma (1). Ptosis occasionally accompanies such pupillary dilatation, but other signs of third nerve impairment are uncommonly observed—probably because their later appearance in the course of progressive tentorial herniation is masked by coma.

The two patients with subdural hematoma and secondary tentorial herniation reported here are exceptional in presenting with major oculomotor nerve palsies with normal pupils.

Case Reports

Case 1

A 30-year-old woman with a history of chronic drug abuse and seizures was admitted in stupor with the diagnosis of drug overdose. No evidence of trauma was apparent and toxic serum levels of barbiturate and Dilantin® were present.

Two days after admission she was sufficiently alert to complain of headache and was noted to be moving her right extremities less than the left.

Neurologic evaluation on the sixth hospital day demonstrated a lethargic woman with a dense right hemiparesis and a moderate right-sided decreased sensation to pin. There was complete right ptosis and right eye motion was limited to a few degrees downward (Fig. 1) with full lateral movement. Left eye movement was normal. The pupils were equal in bright and dim light, and the pupillary light reaction was brisk bilaterally (Fig. 2). The optic fundi and the visual fields were normal.

The cerebrospinal fluid pressure was 90; and the clear, colorless fluid contained 800 red cells, 8 white blood cells (3 neutrophils) and a protein of 28. Skull films were normal; an echoencephalogram demonstrated a right to left midline shift; and carotid angiography revealed a large right subdural hematoma with a medial shift of the anterior choroidal artery indicating uncal herniation (Fig. 3).

All signs slowly cleared following evacuation of the hematoma. One month later the third nerve palsy had cleared completely and a mild right-sided weakness persisted.

Case 2

A 30-year-old alcoholic woman complained of right-sided headache, dizziness, vomiting, difficulty in walking, and double vision on the day following a fall down stairs without loss of consciousness.

On examination three days after the fall she was mildly lethargic and exhibited a right

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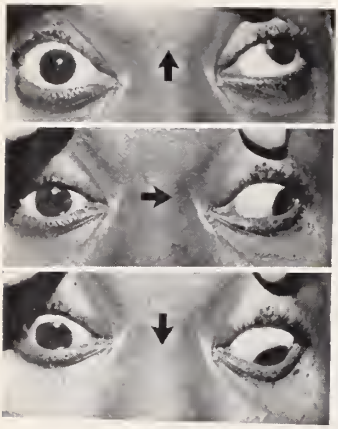

FIG. 1. Case 1: Gaze up, left, and down showing right eye limitation. Pupils are equal.

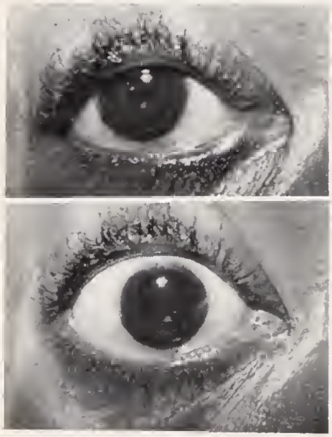

FIG. 2. Case 1: Right pupil under bright (top) and dim illumination demonstrating normal light reaction.

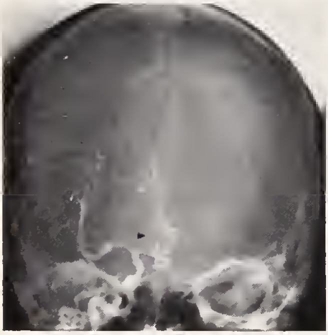

FIG. 3. Case 1: Frontal view of right carotid angiogram showing medial displacement of the anterior choroidal artery (arrow).

frontal bruise. The right lid was moderately ptotic and the movements of muscles innervated by the right third nerve were limited to less than one-fourth of their normal range (Fig. 4). The pupils were each 4 mm in size and reacted briskly and equally to light. The remainder of the neurologic examination was normal aside from a mild nonspecific gait ataxia.

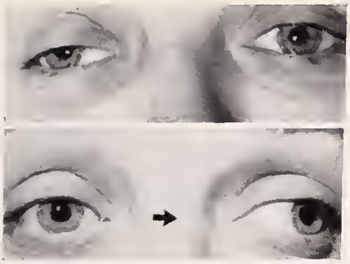

FIG. 4. (Case 2: Forward and left gaze showing right ptosis, marked right globe limitation, and equal pupils.

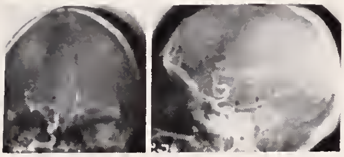

FIG. 5. Case 2: Frontal and lateral views of a right carotid angiogram demonstrating downward (double arrows) and medial (single arrow) displacement of the posterior cerebral artery.

Skull x-ray films were normal and a lumbar puncture revealed grossly bloody fluid with a pressure of 260. A right carotid angiogram demonstrated a large subdural hematoma and swelling of the temporal lobe with medial and downward displacement of the posterior cerebral artery indicating herniation along the entire length of the tentorial opening (Fig. 5).

On the third day following hematoma evacuation only a trace of third nerve paresis remained and all signs cleared within one week.

Discussion

Major involvement of the third nerve is rarely observed in tentorial herniation. Pevehouse and associates (1) in a review of the eye signs in 389 cases of subdural hematoma reported 116 instances of pupillary inequality, but only 18 of these patients demonstrated other signs of third nerve dysfunction (usually ptosis), and complete paralysis was observed in just three cases.

Govan and Walsh (2) studied 37 infants and 54 adults with subdural hematoma and found no examples of complete oculomotor nerve palsy and only three instances of ptosis with homolateral pupillary enlargement.

In a series of 87 cases of epidural hematoma with ipsilateral pupillary involvement, only nine patients exhibited external ophthalmoparesis, and the only two cases with total oculomotor paralysis had extensive orbital hematomas (3).

Four of Kaplan's eight cases of chronic subdural hematoma exhibited fragments of third nerve dysfunction aside from pupillary dilatation (4).

The two cases reported by Clark and Gooddy (5) are of particular interest. The first presented with a complete oculomotor nerve palsy ipsilateral to an acute subdural hematoma, but recovery of both pupil and extraocular muscle function began on the next day. By the third day there was only a trace of palsy which persisted until the patient's death from tentorial herniation eight days later. Their second patient entered with a "50%" third nerve palsy associated with a slightly smaller and briskly reactive pupil. The oculomotor palsy slowly improved and the pupil remained smaller. Following surgery, the diplopia disappeared but the pupil remained smaller and was noted to be less reactive than its fellow.

The precise mechanism of pupillary dilatation and extraocular muscle paresis in tentorial herniation remains in doubt but experimental and pathologic evidence favors direct compression of the peripheral third nerve rather than midbrain compression. The clinical observation of a fixed dilated pupil preceding dysfunction of globe movement has been experimentally reproduced in animals by inducing tentorial herniation with ipsilateral expanding supratentorial masses (6, 7). Oculomotor nerve fiber transmission is blocked at the time of third nerve dysfunction with herniation (7). Direct compression of the third nerve has been shown to produce mydriasis whereas midbrain tegmentum compression results in miosis (8).

Smaller diameter nerve fibers occur peripherally in the third nerve—especially supero-medially (8, 9)—and are thought to represent pupilloconstrictor fibers on the basis of sectioning and stimulation studies (8). The demonstration that the nerve infarct producing a diabetic pupil-sparing third nerve palsy is restricted to the central portion of the nerve (10) further supports a pupillary function for the small peripheral fibers. Pupillary fibers are said to be more physiologically sensitive to compression; however, prompt pupillary recovery after decompression is difficult to reconcile with this suggestion (11). An alternate hypothesis explains early pupillary dilatation in herniation as the result of specific involvement of the supero-medial portion of the nerve by the downward-displaced posterior cerebral artery (11). Initial pupillary constriction has been observed clinically in herniation and attributed to "irritation" of the autonomic fibers but experimental studies have not consistently duplicated this miosis (8).

The present cases demonstrate that the occasional major third nerve palsy observed with tentorial herniation may have completely normal pupillary reactions. A number of observers (3, 7, 11) have noted rapid recovery of the dilated and fixed pupil in tentorial herniation following relief of pressure, and marked improvement of a third nerve palsy has occurred in the presence of continued herniation (5). Our cases most likely represent instances of partial relief of third nerve compression despite continued herniation with recovery of pupillary function but persistence of marked extraocular muscle weakness.

Summary

Two patients with subdural hematoma exhibited major oculomotor nerve palsies with complete pupillary sparing. Angiograms demonstrated tentorial herniation in both patients. Neurologic signs cleared following hematoma evacuation, and the oculomotor palsies resolved completely.

A marked third nerve paresis is seldom seen in association with tentorial herniation and pupillary sparing in such instances is quite rare. The normal pupils probably represent partial recovery of third nerve compression despite continued herniation.

Acknowledgments

Michael Devereaux, M.D. photographed the first patient; James Teal, M.D. assisted with x-ray interpretation.

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Newer Aspects of Brain Scanning at Mount Sinai

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Since the introduction of short-life isotopes for medical diagnostic purposes, the brain scan has become an integral part of the neurologic examination. The medical approach to the treatment of neurologic disorders as emphasized by Dr. M. B. Bender at Mount Sinai has been particularly instrumental in fostering the growth of nuclear medicine as applied to neurology. This has permitted the use of scanning, not only as an initial diagnostic tool, but also to follow patients over months and years and to observe changes that have not been altered by surgical intervention.

We will describe the more recent developments in brain scanning and some of our observations with the use of these newer techniques. Some of these observations are similar to those made at other institutions. Others, particularly those related to nonsurgically treated subdural hematomas, are unique.

Serial Scans after the Initial Injection of Isotope

The type of scan referred to as rectilinear is performed by placing a gamma detector over the patient's head after an intravenous injection of technetium pertechnetate ($Tc99^{m}$). The detector is small and must be passed back and forth over the patient's head until all areas have been described. The activity noted in the sampled areas depends upon the amount of radioactivity detected by the probe and is recorded by exposing photographic film with proportional amounts of light.

A second type of scanner, the gamma camera, is large enough to view the entire head at the same time. Once positioned, it remains fixed. The activity detected is shown on an oscilloscope and a permanent record is made by photographing the screen.

Experience with static scans has shown that lesions are best visualized at about one to 1.5 hours after injection. Certain lesions however, such as infiltrating brain tumors, appear to take up the isotope less rapidly (1). If suspicion of such a lesion exists, the serial scan is done at the conventional time and then repeated one to two hours later. An example of this, where visualization is initially negative but becomes positive after the longer time period, is shown in Figure 1.

The Brain Scan in Short- and Long-Term Follow-up

A. *Short Term.* The interpretation of a static brain scan is based on the visualization of isotopic uptake in an area where it should not normally be,

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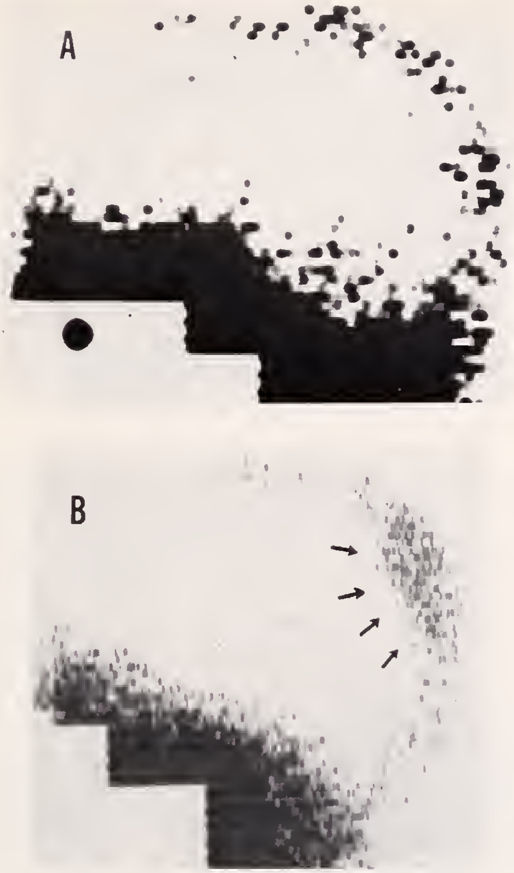

FIG. 1. Patient with right hemiparesis and right visual field disturbance. a) Left lateral Tc99m scan performed at 1 hr after injection. No lesion is detected. b) Left lateral scan performed the same day at 3 hr shows a left occipital lesion.

the intensity of this uptake, and its geometric shape. Certain lesions, such as meningiomas, seem to have a characteristic pattern and can be interpreted with statistical accuracy (2).

More often, it is difficult to be definite about the nature of the lesion, for example, in differentiating between an infarct and an infiltrating brain tumor. Diagnosis is established by performing sequential scans over a period of weeks. Infarcts are frequently not present on scans done a few days after the onset. They usually become positive after a week and tend to disappear over two months (3). An example of this is shown in Figure 2. Primary brain tumors tend to persist or enlarge during this period (4, 5). Metastatic lesions tend to persist, enlarge, or show additional new lesions (6).

B. Long-term follow-up. Static scans on patients with nonsurgically treated subdural hematomas, some of whom were followed for as long as four years, show that the scan gradually improves as the subdural resolves. This is con-

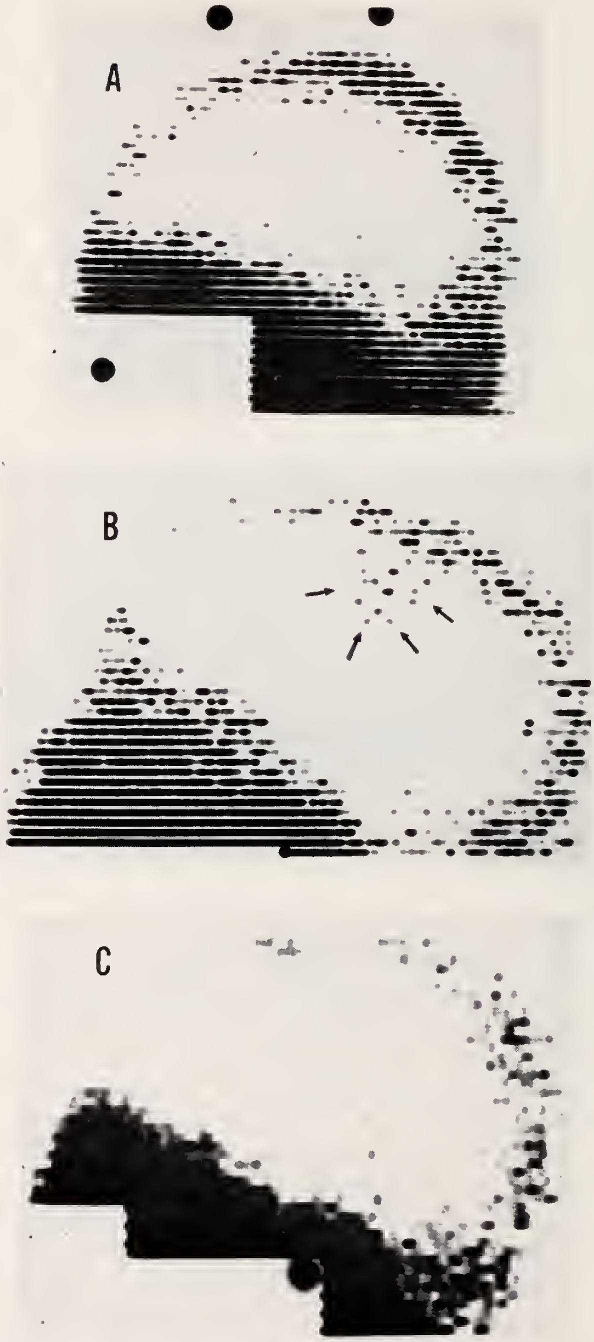

Fig. 2. 64-year-old woman presented with acute onset of right hemiparesis and mild aphasia. a) Left lateral scan performed at 48 hours after onset of illness, scan is normal. b) Left lateral scan done 8 days later shows a triangular parietal area of increased activity as outlined by arrows. c) Left lateral done at 6 weeks after admission now is normal.

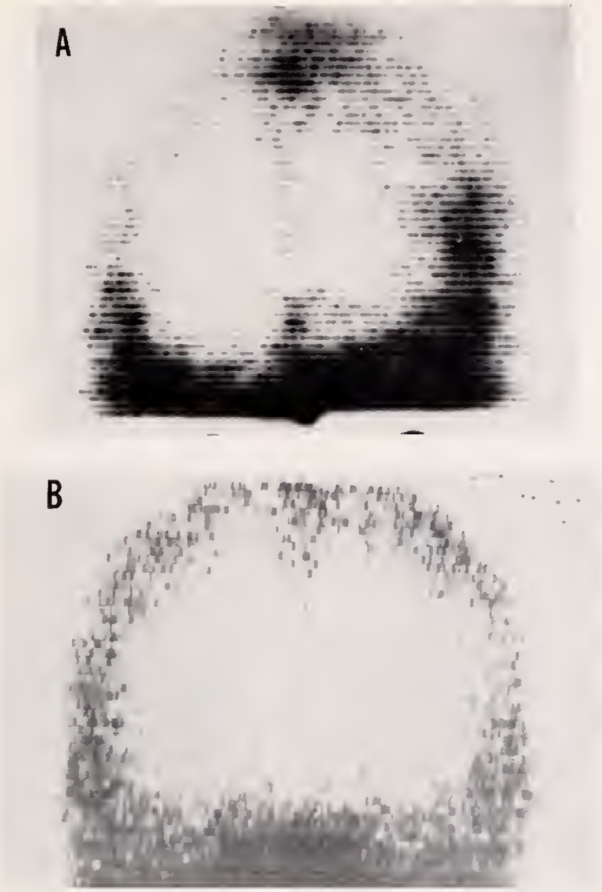

FIG. 3. Patient with right hemiparesis and depressed level of consciousness. Left subdural on angiogram. a) Tc99m scan, anterior view, showing a peripheral crescent on left compatible with subdural. b) Three years later—patient not operated, no neurologic deficit. Anterior scan normal.

trary to surgically treated patients where the scan may remain positive after the subdural has been removed (2) (Fig. 3).

Dynamic Brain Scans

Dynamic brain scans are performed under the gamma camera by injection of a bolus of the isotope ($Tc99^m$) into a cubital vein and recording the initial transit of the isotope through the cerebral circulation (7). During this transit, the technetium stays within the vascular compartment. The passage is recorded on video tape and processed by a dedicated application minicomputer. The transit is analyzed in serial static frames of a fixed duration, usually one second. Each frame may also be projected on a CRT display and visually analyzed. Distinct regions of interest can be outlined and by computer analysis the amount of isotope in that particular area can be determined for that particular frame or the whole study as well. This is useful in comparing flow in symmetrical areas such as the carotid arteries or occipital lobes (7). An example of an abnormal flow pattern is illustrated in Figures 4 and 5.

One application of dynamic scanning particular to Mount Sinai is the following of nonsurgically treated subdurals. The patients are scanned initially at two- to three-week intervals and later at intervals of months. Although

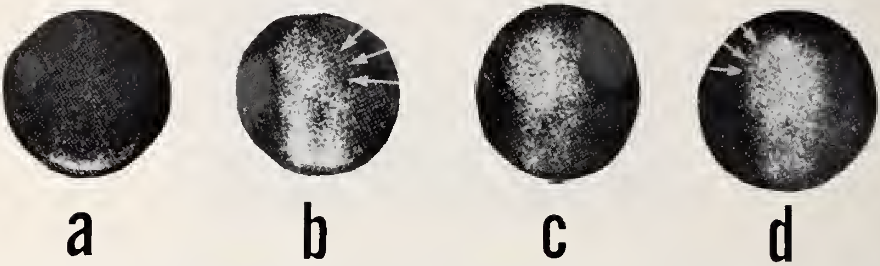

Fig. 4. Flow study with Tc99m. a) At 1–2 seconds, early bilateral carotid filling is noted. b) At 3–4 seconds, both carotids are well filled, a left-sided delay in filling is outlined. c) 6–8 seconds later, left side is becoming perfused. d) At 10–12 seconds, right side now has less perfusion than left. At this time, both sides should be clearing. Therefore, the activity on the left is abnormal and is compatible with delayed flow noted in vascular occlusive disease.

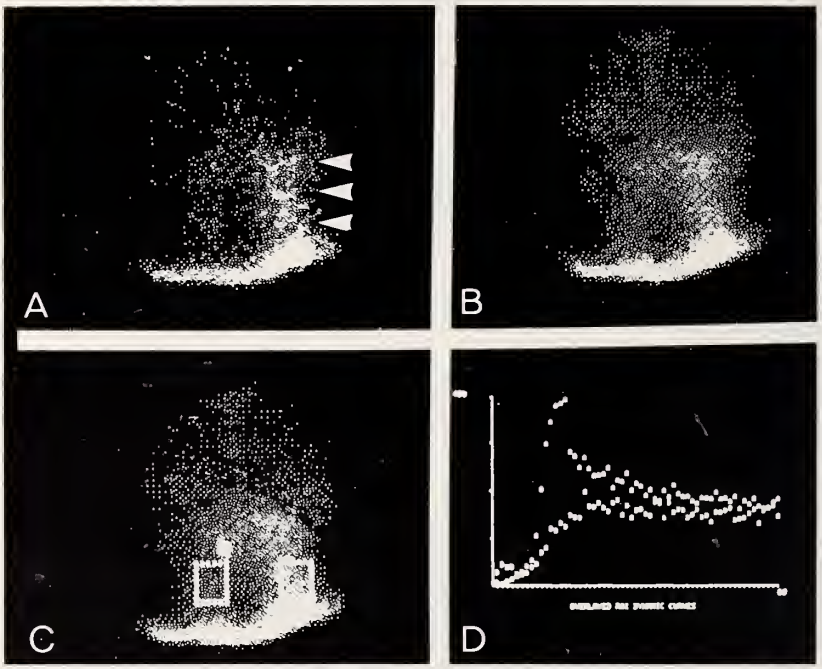

Fig. 5. Patient with an arteriovenous malformation involving the left internal carotid and jugular vein. a) Shows increased flow in area of malformation. b) Illustrates filling beyond the malformation. c) Equal-sized regions of interest are outlined over both carotid arteries. d) Flow through both regions of interest are plotted in an overlay graph over a 60-second interval. Graph illustrates that there is significantly more flow through left carotid as compared to right.

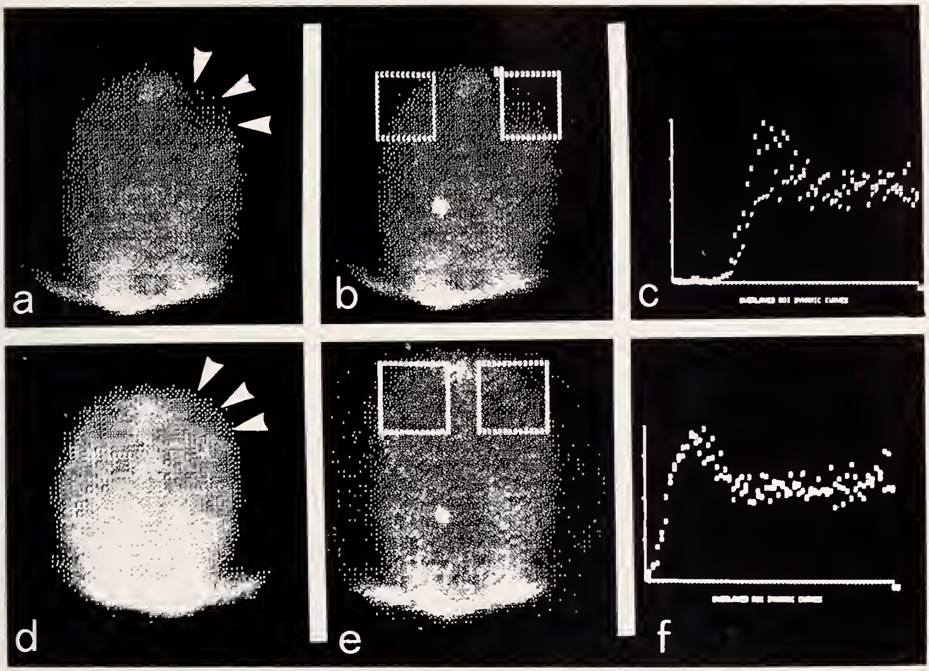

Fig. 6. A 68-year-old male with aphasia, mild hemiparesis. Angiogram showed left subdural hematoma. a) Anterior flow study showing a concave defect in perfusion on left as outlined by arrow. b) Areas of interest are outlined on both hemispheres. c) Overlay graph illustrated increased flow through area of interest on left as compared to right. d) 6 weeks later—patient returns as outpatient, anterior view of flow study no longer shows the previously noted defect. e) Regions of interest are outlined again. f) Overlay graph of flow over 60 seconds shows that flow is now the same on both sides.

our series is still relatively small, we have already shown that the crescent-shaped subdural avascular defect disappears. As our series enlarges, we may be able to eliminate the need for repeated angiography after the initial angiogram is performed to outline the subdural (Fig. 6).

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Carbohydrate, Lipid and Energy Metabolism in Brain Development

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Probably the most complex process in biology is that in which the genetic information in the fertilized egg is utilized to produce a mature brain. A major area of research of the Division of Neurochemistry of the Mount Sinai Department of Neurology has dealt with the production and utilization of energy in the developing brain and the effects of several environmental influences on development. In tribute to Dr. Morris B. Bender, whose inspiration and support in the establishment of this Division has provided nurture for our work, we will review the Division's contributions to the study of developmental brain chemistry.

Although differing in duration, timing and extent, the patterns of changes in form, chemistry and function are similar in all mammalian brains from mouse to man. Development is controlled by a hierarchy of environmental influences: cell-to-cell interactions (transport of material between cells by transient or permanent connections); the extracellular environment within the central nervous system; nutrient supplies, electrolyte gradients, hormones, and specific growth factors; and, after the establishment of functioning sensory organs, factors external to the organism which affect maturation and learning.

Before taking up a detailed description of metabolic events, a word on methodology is in order. In biochemical work it is customary to express the concentrations of chemicals per gram of wet weight or per gram of protein. However, during brain development profound and nonparallel changes occur in intra- and extracellular water, protein, lipid content, and cell concentration; and apparent patterns of development differ depending on how data are expressed. For instance, since the brain becomes much drier during later development, the concentration of a substance may show no change when expressed per gram of protein, yet increase significantly when expressed per gram of wet weight. Failure to comprehend such factors may obscure significant changes during growth or exaggerate trivial ones. Another methodologic problem derives from the fact that different regions of brain are themselves heterogeneous. Even the neuron, although a single cell, is a complex of organelles and specialized elements such as neurofibrils and synaptic structures. Different regions, and to a lesser extent different structures within a region, mature at different rates and studies of major brain parts such as the cerebellum may obscure significant

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changes in specialized regions (1). In our own work we have made use of microchemical techniques to study regions such as the layers of the cerebellar cortex which are naturally enriched in dendrites and synapses, neuronal cell bodies, or myelin. We have also studied the biochemical changes occurring in myelinating tissue cultures of rodent brain or spinal cord material in collaboration with Dr. Murray B. Bornstein.

1. Phase of cell multiplication: During the first phase of brain development almost all the cells which will be adult neurons and most of the glia are formed. At the end of this phase, the DNA content of the brain approximates that of the adult. In general, as with the other phases of development, this occurs earlier in the more caudal regions and later in the cerebral and cerebellar cortices. However, the phases of brain development overlap; and small neurons and glia continue to multiply well into the second phase and to a small extent beyond. Almost all of the primitive neurons migrate to the periphery of the neural tube (making a "U" turn) and also may travel along and across the neural axis (2). At this stage the neuronal processes have not yet formed; thus, the enormous expansion in the membrane of these processes has not yet taken place and the levels of the largely neuronal membrane lipids, such as gangliosides are very low (3, 4). The extensive membrane surface of the adult brain takes up space largely at the expense of tissue water; and before it has formed, the water content is much higher than it will be later. As the brain matures, its dry weight (expressed as a fraction of wet weight) doubles. The brain at this stage is largely a suspension of cells; and the ratio of extracellular to intracellular space and electrolytes is higher than it will be later. Thus the sodium:potassium ratio is relatively high, as it is in the extracellular space. The energy requirements are quite low and the brain is poorly vascularized both in capillary penetration and blood flow. The low energy requirement is not due simply to incapacity to produce energy rapidly (although this is true), but it is also a consequence of a morphologic and functional state that requires little energy. The level of glycolysis is low and that of tissue respiration even lower. Our measurements of several enzymes of carbohydrate metabolism in rodent cerebrum and cerebellum beth in situ and in tissue culture (5, 6) reflect this state of affairs.

2. Phase of neuronal growth and synapse formation: Although most of our detailed knowledge of brain development is based on rodent brain, the evidence indicates that human brain follows a similar pattern. The degree of brain development at birth varies among mammalian species. In the sheep and guinea pig the brain is relatively far advanced at the time of birth and the neonate is able to walk almost immediately. In contrast, the rodent and human brains are quite immature at birth. The rodent brain is more immature than the human, resembling a human brain in the sixth to seventh month of fetal life. Animals such as the human and rodent have an advantage at birth. Because of the low energy requirement of their immature brains they are better able to sustain periods of anoxia associated with parturition. The major morphologic change during the second phase of brain development is the growth of cell processes (dendrites and axons) and the establishment of synaptic contacts (7). The patterns of interneuronal connections appear to be established either by the outgrowth of axons "searching out" by unknown (probably chemotactic) mechanisms their proper target cell (8) or by the migrating neurons establishing contact in passing and then playing out their axons as they continue to migrate, thus maintaining their original contact (2). For example, the granule cells of the cerebellum form their Purkinje dendrite connections during their inward migration from the primitive external granular layer (2, 9). Lipids of high concentration in neuronal membrane (gangliosides) reach adult levels during this period (3, 4) and there is a rapid decrease in tissue water content and increase in intracellular space.

Neurons retain their excitable state by maintaining an electrical gradient across their membranes. A key element of the pump mechanism which maintains the ionic gradient is the enzyme Na^+-K^+ stimulable ATPase (10). The activity of this enzyme increases in parallel with the increase in the bulk of excitable neuronal membranes. Enzymes associated with the manufacture and destruction of neural transmitters and the levels of the transmitters themselves increase concomitantly with the number of demonstrable synaptic contacts. Such processes as maintenance of ionic gradients, synthesis and reuptake of transmitters, axonal growth, transport maintenance and, in white matter, growth of the myelin sheaths as extensions of oligodendrocytes, require an enormous amount of energy and are primarily responsible for the consumption of more energy per gram by the adult brain than by any other tissue except actively contracting muscle. The metabolic machinery which supplies energy develops concomitantly with those structures whose function increases energy demand. At the beginning of the stage of neuronal development the energy needs of the tissue could easily be met by glycolysis, a process which yields only about 6% of the total energy available in glucose, the major brain fuel. At this period there is relatively little complete oxidative metabolism of glucose (glycolysis plus respiration). As the energy demands of the brain increase there is an increase in enzymes which sustain ATP levels such as creatine phosphokinase (11) as well as in glycolytic enzymes which metabolize glucose to pyruvate in the cell cytoplasm (5, 6, 12). Even with this increase, the energy produced by glycolysis is insufficient to maintain neuronal function in the mature brain, as studies of hypoxia have shown (13). Surpassing the increase in glycolytic metabolism during phase two are changes in the size and structure of the mitochondria, the cellular organelles which contain the enzymatic equipment for the oxidation of the acetate derived mainly from glucose, which enable the cell to extract 16 times more energy from a glucose molecule than is provided by the glycolytic process (12, 13). All enzymes and cofactors directly associated with mitochondria increase tremendously during this period as do the enzymes in the cytoplasm which transfer hydrogen into mitochondria and those glycolytic enzymes attached to the mitochondria (5, 6, 14, 15). The supply of nutrients and oxygen for energy metabolism requires increased vascularization of the brain during this stage and there is indeed a great increment in capillary density and regional cerebral blood flow. By the end of this phase of development the brain is metabolically almost mature, consumes about one-fifth of the body basal oxygen requirement and is, henceforth, extremely vulnerable to decrease in oxygen or blood supply or to respiratory poisons. These changes in energy metabolism during development occur also in isolated fragments in organ culture. We have examined the biochemical changes occurring in newborn rodent tissue grown in organ culture by Dr. Murray B. Bornstein, Albert Einstein College of Medicine (ref. in this issue). Not only do total enzyme activities in these cultures increase in parallel to those in the living animal (5, 16–17), but the pattern of proteins with a given enzyme activity also changes in parallel in neural tissue developing in vivo and in vitro (14, 18).

The major morphologic and biochemical changes associated with neuronal process growth and synapse formation are completed during a short portion (roughly one-thirtieth) of the total life span of mouse or man. Although modifications associated with learning continue throughout life, no corresponding changes in lipid or energy metabolism have been observed. Contrary to previous concepts, we found significant biochemical changes in the mouse cerebellum occurring after the supposed completion of phase two of development—when the cerebellar cortex is morphologically mature (9) and after the young animal's motor capacities have apparently matured. This period may be designated phase 2b to distinguish it from the earlier rapid biochemical changes which have clear-cut structural parallels. Because these changes are localized to specific cortical layers, they would be obscured in analyses of whole cortex. They are particularly marked in the neuropil. For instance, in the synapse-rich molecular layer of mouse cerebellum after 30 days of age, there is a distinct decrease in cell density without change in the lipid content (19). There is no visible myelin in this layer and little myelin associated lipid (20). The decreased cellularity is thus most likely related to separation of cell bodies by nonmyelin cell processes—i.e., neuropil (the complex of dendrites and axons which comprise the synaptic structures). Despite the decline in cellularity there is a highly significant increase in enzymes of several pathways of energy and carbohydrate metabolism (1, 21, 22). Changes under stress in the rates of turnover of carbohydrate and energy rich intermediates (e.g., ATP) in the molecular layer during development have also been measured (21). Since the cellularity of the cerebellar molecular layer decreases during this period, the added metabolic capacity must be a property of the neuropil. The structural and functional changes underlying the enzymatic changes of late development are not clearly documented, but their magnitude and specific histologic localization speak for their significance. It is possible that they are, at least in part, related to the continuing modification of neuropil associated with motor learning.

3. *Phase of myelination:* The final stage of brain development is related to the myelination of axons. This occurs at different rates in different regions. It

begins during the stage of neuronal outgrowth and ends anatomically after maturity is attained. In the human, myelination continues into the third decade. Although the layers of modified oligodendroglial cell membrane which constitute central myelin are relatively stable, there is evidence for slow, continued turnover of myelin constituents throughout life, i.e., the constituents of myelin are thus both manufactured and broken down. In general, hereditary diseases associated with metabolic defects in breakdown of myelin lipids (e.g., metachromatic leucodystrophy) present clinically later in life than those associated with mainly neuronal lipids (e.g., Tay-Sachs).

During the early period of myelination oligodendrogliocytes (and astrocytes) migrate to their final positions and differentiate; and the oligocytes enwrap the axons; this is accompanied by accumulation of myelin lipids (3, 4). If antibodies active against myelin are added to the media of normally developing organ cultures of embryonic spinal cord, the formation of certain myelin lipids, oligodendrocyte differentiation, and myelination either are prevented or halted simultaneously (23, 24). Later during phase 3, myelin is added mainly to elongating axons; therefore, the lipid concentration in a given volume of white matter remains constant, while the total volume of white matter increases (25). Because of this white matter expansion, the (glial) cell density within the white matter declines sharply (19). Concomitant with the onset of myelination there is an increase in the activities of several enzymes associated with lipid metabolism in general and with that of myelin lipids in particular (5, 6). The patterns of change of these enzymes differ from those of the general metabolic enzymes which usually reach a plateau sustained through young adulthood. These myelin-associated enzymes reach a peak during the period of most active myelination and decline to sustained levels well below their maximum activities when myelination is completed (5, 6, 21).

The metabolic rate in adult white matter is only about half that in average gray (21) and the regional blood flow and capillary density are comparably lower (26). However, the myelin membranes take up a large volume and reduce the protoplasmic mass. If one refers the biochemical measurements to intracellular (metabolically active) portions of white and gray matter by basing the data on lipid-free dry weight or soluble protein content, then it appears that the enzyme activities and the metabolic rates are surprisingly high in white matter (21, 22). Expressed in this way, both enzymes and energy utilization rates in cerebellar white matter approach those in the neuropil-rich molecular layer and exceed those in the granular layer (composed mainly of densely packed cell bodies). The changes with maturation suggest that the high metabolic capacity of the white matter is related to axonal transport and maintenance of myelin. Following the completion of active myelination, the activities of several enzymes related to energy metabolism and the metabolic rate of white matter slowly decline below their levels during myelination .(21, 27). The blood flow to white matter also declines after the completion of myelination (28). The higher energy requirement of white matter during myelination may contribute to the greater vulnerability of immature white matter to asphyxia and ischemia relative to that of the adult. However studies of the changes in the energy reserves during ischemia indicate that there are at least two major metabolic compartments in white matter (21, 27). One is responsible for the high steady-state (living) energy metabolism of the nonlipid regions of the white matter. The other is capable of sustaining its energy reserves longer during ischemia. It may be that the rapidly metabolizing compartment is associated with axonal transport and shares the neurons' vulnerability to hypoxia. The second is probably glial and is responsible for the relative structural resistance of adult white matter to hypoxia.

In conclusion, brain development is characterized by a well-defined sequence of changes in structure, function and metabolism. Using the rodent as a model we have accumulated sufficient data to evaluate the molecular effects of various experimental manipulations on brain development both in vivo and in tissue culture. Currently this approach is being applied to the investigation of immune mechanisms by which sera from patients with multiple sclerosis and from animals with experimental allergic encephalomyelitis prevent myelination and disrupt myelin (23, 24); the retardation of brain devlopment by quantities of lead that do not produce acute encephalopathy (29) and the long-term effect of exposure of pregnant mice to marijuana constituents on brain development in their offspring.

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On the Significance of Breaking Symmetry for Normal Behavior

With Particular Reference to Motor Performance

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Foreword in Honor of Dr. Morris B. Bender (MBB)

I opposed the titling of this issue Festschrift since the word 'festschrift' does not apply to MBB. It may have applied to the "giants" of the past—the amerikanischen Herren Professoren. MBB is not Herr Professor. He earned his respect through his competence that borders on genius: from the unique way he discovers the "twist" in a problem that no one else can see; from the way he instills a freedom of expression and the desire to challenge the obvious—the dogma of the day; from his "open" conferences where the intern or medical student is invited to interrupt, even in the middle of a sentence, to clear up or challenge the immediately preceding statement, while it is still in context, rather than later when the statement and question may have become vague and stale.

Grand Rounds on Friday afternoons, for me at least, serve as a mental purge. It is a forum to state, argue, challenge, defend, or even abandon a point of view in neurology. A typical Grand Rounds resembles a free-for-all debate in the House of Commons. To the visitor or invited guests, it appears that MBB had lost control of this unruly "mob." However, one soon finds himself entering into the fray and realizing it is intellectually healthy, despite the fact that he might now be confused about all his previous concepts. This is one place where anxiety is beneficial. The arguments, or heated discussions, if you will, always are related to the subject matter and never intended to relate to the individual.

It is this spirit of free, open expression that attracts so many young people to the field of Neurology at The Mount Sinai Hospital and to MBB in particular. He plants the seed of curiosity and enthusiasm and inculcates in many a fresh approach to the investigation of human behavior. It is no wonder that in the last twenty-five years he trained several hundred people who remained in the field of Neurology rather than branch off into Psychiatry or Neuro-

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surgery. Many are now researchers of the highest caliber in universities throughout the United States and foreign countries, and a good number are professors and/or chairmen of their departments. Not all pursued the same interests as Dr. Bender, but all were infected by his free and open-minded approach in dealing with problems.

"The patient is correct, the book is wrong." "I am just a boy, I am learning." "To those who know, good for them. I'm still searching." These are some of the statements that are familiar to those who have had contact with MBB.

This attitude, this enthusiastic drive, this approach, is what makes one neurologist recognize another neurologist as having been trained by MBB.

MBB is a gentle man, without the gentility expected of members of a university faculty. He remains a rough diamond that resists cutting and polishing.

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The human brain is a symmetrical organ with mirror image structures, and yet the average healthy individual is able to perform asymmetrically. He can move one thumb without moving the other; he can write; he can speak; he can create asymmetrical patterns; he can play the piano striking notes with the fingers of one hand while he is or is not striking different notes with the other. Typing is another good example of asymmetrical performance.

Despite the anatomic symmetry of the brain, functional differences occur during the course of normal development so that each hemisphere becomes more independent and subserves different functions (1). Symmetry must be broken to allow the normal person to carry out the chores of daily living.

There is much evidence that symmetry of motor performance remains as the underlying tendency in all normal subjects (2–4). This basic strong tendency for symmetry lasts throughout the life span of man. During his childhood years, the breaking of symmetry is essential and proportional to his motor development. His ability to perform alternately and later (2, 3, 5, 6) to master purposeful, unilateral, discrete movements without associated synkinetic movements of the opposite limbs, is necessary for him to walk, run, alternately step up and down stairs, learn to write, or play a musical instrument. Persistent symmetrical movements in the form of mirror movements are seen in extreme cases where symmetry cannot be broken because of "brain damage". Similar reversion to symmetrical movements (mirror) are also seen in adults with acquired cerebral disease. From childhood to senility, the average "normal" individual will break symmetry to varying degrees of proficiency from the clumsy "all thumbs" to the "talented".

Investigation of motor performance by special tests in normal adult subjects has been carried out to determine the ability to (a) cross the midline, (b) perform ipsilaterally and contralaterally, and (c) to evaluate how strong the basic tendency is to symmetrical performance. In 1956, in a study titled *Phenomena of the Inability to Cross the Midline in Patients with Cerebral Dysfunction* (5), it was found that 20% of the patients with organic mental syndrome without aphasia or hemiparesis found it difficult, or could not, cross the midline on command. For example, to the commands "put your right hand on your left ear" or "touch your right ear with your left hand" or "touch your right eye with your left hand" or "touch your left eye with your right hand", the responses were right hand to right ear, left hand to left ear, right hand to right eye, and left hand to left eye. These patients also found it difficult to cross the midline even when requested to imitate the examiner. For example, the examiner rubs his right ear with his right hand and the patient rubs his right ear with his right hand. Examiner then rubs his right cheek with his right hand and the patient rubs his right cheek with his right hand. Examiner rubs the tip of his nose with his right hand and patient rubs the tip of his nose with his right hand. Then, examiner rubs his left ear with his right hand, but the patient returns to rubbing his right ear with his right hand.

Later, in 1959, this problem was approached from the point of view of contralateral versus ipsilateral motor performance in normal subjects (2). One thousand normal adult subjects, ages 16–78, and five hundred normal children, ages 3–14, were surveyed. It was learned that with simple commands requiring ipsilateral performance such as "touch your ear"; "touch your knee"; "touch your hip"; etc., the tendency was overwhelmingly that of ipsilateral performance, 90%. Of course, with the command "touch your elbow," all were correct but many hesitated attempting to touch the ipsilateral elbow and then touched the opposite elbow. When commands were given requiring contralateral performance, 95% were correct after the third trial. The older group, ages 65–78, and the children, ages 4–14, made most of the errors in this first two trials. For example, the subject is requested to place the right thumb on the left ear or left hand on the right eye.

The difficulty in crossing the midline, or contralateral performance, in patients with cerebral disease was previously described.

These studies gave us information about ipsilateral and contralateral performance in normal subjects. But what about bilateral simultaneous performance in normals? It is well known that most "normal" people have difficulty in carrying out two different types of motor performance simultaneously. This led to another test that could be easily demonstrated and documented and was named "The Imaginary Piano-Finger Test" (3). Normal subjects, ages 6–85, were asked to place their hands on a table and imagine it was the keyboard of a piano. They were requested to strike random notes rapidly with the fingers of both hands on this imaginary keyboard. At varying intervals they were given the command to "stop", and the position of the fingers on and off the keyboard was noted and photographed. After five trials, the subjects were directed to give themselves the command to stop for five more trials. This investigation included subjects from all walks of life and persons with different degrees of dexterity, such as musicians, typists, and those involved in skilled manual trades. Ninety-one percent (91%) of the normal subjects investigated stopped on symmetrical fingers or stopped with all fingers off or all fingers on the keyboard on every trial including those when the subject gave himself the command to stop. Ninety-eight percent (98%) of this group made symmetrical fingers stop on seven out of ten trials. Only 2% made more than five asymmetrical stops and more than 60% of these were persons with high degrees of manual dexterity.

In addition, of fifty patients with diffuse cerebral disorders without aphasia or hemiparesis, all showed symmetrical stops and in 75% of this group the stops were with all fingers *off* or all fingers *on* the board.

As was already mentioned, the reversion to extreme symmetry of performance may occur after symmetry has been broken, due to cerebral disease. Two examples follow.

Case 1: According to what was told to him by his parents, a fifty-year-old man, J. D., had been well and developing normally until the age of five when he had a sudden severe right hemiparesis during the course of "meningoencephalitis." After a number of years he subsequently learned to walk and had several operations on the right wrist and forearm to correct deformities that developed. At no time was his speech impaired nor did he have difficulty with language communications. He stated that at about the age of twelve or thirteen he realized that if he wanted to move any finger of the right hand, the same finger on the left hand also moved and that this was beyond his control. In addition, he realized that every time he moved a finger on his left hand (the "good" hand) there was simultaneous movement of the same finger on the "bad" right hand. This was also true when attempting to open and close either hand; they always performed simultaneously and symmetrically.

Case 2: A sixty-four-year-old man, I. M., gave a background history of diabetes for at least thirty years which had been difficult to control, and he had several episodes of hypoglycemia and one period of diabetic coma in 1954. In addition, he had bilateral iridectomies for cataracts in 1953.

His recent neurologic symptoms and signs began in March 1972 when he was admitted to another hospital for sudden left hemiparesis. In addition to the left hemiparesis, a left homonymous field defect was found. The left hemiparesis cleared in several weeks but there was some residual left homonymous field defect.

On October 4, 1972, the patient was admitted to the Long Island Jewish-Hillside Medical Center because of uncontrolled diabetes, anemia and mild congestive failure. These were corrected. However, on the sixth hospital day he developed a right hemimotor disorder. Examination revealed a severe right hemisensory impairment, particularly of the right hand with absent position sense and astereognosis, a mild right hemiparesis including the face, and a coarse nystagmus on both lateral gazes. He was not aphasic. The residual left Homonymous field defect also was demonstrable. Two days later it was noted that the patient had very obvious mirror movements of the fingers and hands. If the command was given for him to make a fist with his right ("bad") hand only, he simultaneously made a fist with his left ("good") hand. If he was asked to move one finger or several fingers of his right hand, the same fingers would move on his left hand. Conversely, any command for motion of his left ("good") hand or fingers was reflected by mirror movements of his right ("bad") hand. Despite the fact that he was asked to please not move "the other" hand or fingers, the movements occurred. When large familiar objects were placed in his right hand and he was asked to feel and identify them, the exact manipulating movements made with his right hand were also made with his left hand. He could not identify the objects. When the same objects were placed in his left ("good") hand, the manipulating movements were the same in both hands, but he correctly identified the objects.

Daily observations were made and the mirror movements persisted even at the time of discharge on November 29, 1972. Although there has been some improvement in his overall condition, the mirror movements persisted as of February 1973.

Motion pictures taken of both cases revealed that the mirror movements at times were less pronounced on the side opposite to the one called for and at other times were even more pronounced. The "good" side and "bad" side were different only by the deformities of the right hand and fingers in case 1 and by the attitudinization at rest of right hand in case 2.

Other studies of the motor functions of limbs opposite to the hemiparetic side have revealed a similar pattern of dysfunction to that of the involved hemiparetic or spastic limbs. Cohn (7) and Hausmanowa-Petrusewicz (8) have referred to these phenomena as interactions between the "good" and the "bad" side, since the "good" side revealed more dysfunction when performance was required simultaneously with the "bad" side. The "bad" side also suffered with double simultaneous performance.

Studies of the gait of spastic hemiparesis (9, 10) comparing both the involved and uninvolved ("normal") limbs revealed similar patterns of dysfunction in the "normal" or "healthy" limbs to that of the involved or "bad" limbs. Also, electrical recordings of the knee jerk on the "good" side revealed a similar, although less pronounced, pattern of dysfunction when compared to the knee jerk of the hemiparetic side (11). Other investigators (12) working with hemiparetics have found that "the demand for bilateral conjoined activity of homologous parts resulted in bilateral reduction in efficiency without the achievement of coordination".

Morrell and Salamy (13) revealed that the average cortical evoked response to natural speech stimuli from electrodes placed symmetrically over the two cerebral hemispheres showed a consistently higher amplitude but similar pattern in the left (or dominant) hemisphere. Reference also is made in this paper to the findings of Bigum, Dustman, and Beck (4), Rhodes, Dustman and Beck (14), and Lewis, Dustman, and Beck (15). They showed that in children, the evoked responses to visual stimuli are greater in the right hemisphere as compared to the left. But, it was also revealed in these studies that this amplitude asymmetry "may be abolished by ingestion of alcohol." Further, these hemispheric differences were *not* observed in mongoloid or mentally retarded children. Once again, this is an example of a return to a failure to break symmetry as reflected by electrocortical responses.

There is further evidence that the strong tendency for symmetry exists not only in motor function but in cutaneous sensory perception and visual perception. Bender in his monograph "Disorders of Perception" (16) has shown that double simultaneous stimulation of heterologous or asymmetrical parts, such as in the face-hand test, elicits significantly more errors (extinction and displacement) than does double simultaneous stimulation of homologous or symmetrical parts, especially in patients with organic mental syndromes.

Symmetry was also found to be characteristic in the phenomenon of allesthesia (17), where a stimulus to one part of a limb is referred to the identical part on the opposite side, i.e., pinprick to the left little finger reported as a pinprick to the same finger of the right hand.

Symmetry in the visual world of the average person is more acceptable or less disturbing than asymmetrical visual stimuli. Perhaps this may account for the early rejection of the works of modern abstract artists. Some people are annoyed if paintings are hung in an "unbalanced" arrangement. The ability to tell the real visual image from the mirror image is difficult for animals and children. The adult has learned other clues in his visual world to make this differentiation (18).

Similarly, I submit that there may be a subjective parallel in the perception of sound. One may be more apt to accept the common tonalities in music and may be uncomfortable hearing discordant sounds such as composed by Stravinsky.

The brilliant studies in physics of Yang and Lee (19) upset the long-standing physical laws of right-left symmetry or the concept of parity of mirror images of matter. The behavioral studies discussed in this essay merely emphasize that symmetrical cerebral hemispheres with their anatomic mirror structures have, in addition to symmetrical functions, different and specific properties. One thing seems clear and it is that in order to fulfill the potentials required for normal behavior, particularly motor performance, the inherent functional differences of each cerebral hemisphere must be realized. Symmetry must be broken.

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An Adaptive Model for Examination of Children with Developmental Problems*

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"We must remember that many doctrines were stated years ago in principle that were then novel and much disputed, but now are so generally accepted that we are in danger of ceasing to think of the very early propounders of those doctrines," so Hughlings Jackson is quoted (1). One may add that not only are early propounders sometimes forgotten, but also the assumptions and context within which they worked. "Such assumptions appear so obvious that people do not know what they are assuming because no other way of putting things has ever occurred to them . . ." (2). The assumptions within which the evaluation of brain function in clinical neurology has developed arose from the constraints established by Hughlings Jackson. His conception was that the brain as an organ was concerned with sensory impressions and motor responses. We as clinical neurologists assume in the classical neurologic examination that all the functions of the central nervous system and their changes under pathological conditions are expressible in terms of excitation (sensory) and reaction (motor), deliberately ignoring the element of consciousness (3).

Nevertheless, it was understood that this application of the reflex arc to explain the function of a living organism is merely a hypothetical construct. We do not believe reflex action to be the true model of the dynamics of an organism unless we conceive reflex action in a much broader framework. Reflex action at any one point in time and place is the outcome of the interaction of that point with the remainder of the organism. "Each reflex must be considered the functional expression not of a single level but of the whole . . . [and] according to a given situation, the past and future of the organism" (4). The perception of sensory stimuli, for example, occurs in the presence of concurrent rather than isolated nervous activity. A so-called pre-potent stimulus affects the very reception of a secondary stimulus by the peripheral sense organs (5).

In a series of clinical studies, Bender and associates have emphasized the clinical value of double simultaneous stimulation (6–9). Such efforts were devoted to localization as the major issue of clinical neurology. Although no structure was considered to be identical with a function, patterns of disturbances in the type and symmetry of performance were used for establishing the site in the nervous system at which a lesion may be expected to exist. In addition, variations in the level of consciousness were considered the context within which such focal disturbances may be elicited. Sodium amytal, for

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example, provided a reproducible means of affecting the level of consciousness leading to more explicit delineation of such dysfunction (10, 11). The face-hand test also served as a clinical tool to illustrate such phenomena. The face seemed to be pre-potent to the dorsum of the hand. Failure to identify the stimuli applied to the hands in the presence of simultaneous stimuli to the face was a measure of general level of consciousness. Failure to identify the stimulus to the hand on a particular side is considered a measure of focal disturbance elicited by the use of such double simultaneous stimulation (12).

A major contribution of such procedures has been a closer approximation of how an organism might actually function. The dimensions added to the model of brain function as reflected in the examination protocol were the effects of level of consciousness and the presence of competing stimuli on perception. In a more general sense there was greater awareness that the results obtained were affected by the conditions under which the organism was examined. The organism was not viewed as defined by a reflex model alone, but as a more nearly integrated whole. The implications of these principles of the integrated organism were, however, mainly harnessed to the localization requirements of clinical neurology.

It is important to recognize the relationship of such clinical evaluation procedures to the specific issues and assumptions of clinical neurology. In the child with developmental problems other issues may be paramount and other evaluation procedures more pertinent. The localization issue of clinical neurology is far less pertinent. In the absence of progressive disease, treatment for the child is rarely that of the physical removal or radiologic destruction of the offending lesion. The concept of a single causative factor whose removal would presumably lead to recovery is also far less pertinent. The underlying cause for developmental difficulties in the child is frequently not amenable to treatment. Indeed the child's function at any one point in time is the result of the interaction of the frequently only putative "damage" by, let us say, perinatal anoxia, and the type and degree of learning which had gone on. Moreover, the potential clearly exists for increased growth and adaptation on the part of the child in the future. The "disease" is not progressive, but the child is developing in the context of the interaction of his nervous system and the environment for his learning. This process of development may be considered as an increase in the degree of organization of an individual considered as a functional adaptive system (13). The clinical protocol for examination in developmental problems must reflect these differences more explicitly.

The Neurodevelopmental Observation

A 15-minute protocol was developed as a model for examination of children with developmental problems. It is called the Neurodevelopmental Observation (NDO). It seeks to illustrate a process of decision-making that may lead to treatment of these problems, just as the clinical neurologic examination has done so for other kinds of problems subject to surgical or other physical methods of treatment. The first assumption that underlies the NDO is that the behavior elicited during an examination is affected by the conditions provided during the examination. If one changes the conditions then one changes within certain limits the function of the organism. The second assumption is that the treatment of developmental problems is not primarily short-term and physical and in the hands of the professional diagnostician. Rather it must be viewed as long term, and in the hands of those who are responsible for the child's continued development. These so-called primary participants are the child, his parents and teachers. The third assumption arising is that the child as well as the primary adults in his environment have a potential for development across time. It is that time parameter that one is sampling in the relatively short-term interaction between these primary participants and the professional diagnostician. Another and even more basic assumption is a broad view of the nervous system. The nervous system is viewed as the physical embodiment of the means by which the organism maintains his- or herself within biological constraints (temperature, etc.) and adapts both to continuity and change in the internal and external milieux (14).

It is such an adaptive process that may be sampled during the time and other constraints under which the diagnostician must work. It is such an adaptive process that must both be simulated and stimulated. The evaluation process is viewed as a dynamic one in which conditions are explicitly varied so that the child's behavior is modified. The objective is both to explore some problem-solving strategies and to explicitly communicate these options to the child and the responsible adults. The clinical examination is a short-term sampling of the change that may occur in the child. The child may be expected to learn something during the examination itself. It is this process of learning that is made explicit and self-conscious.

In the model NDO protocol, a set of prototype tasks is used to illustrate the procedures of this interactive type of evaluation. The first group of tasks is an adaptation from those used in the study of aphasic patients by Henry Head as modified by Benton (15). The eventual goal is to have the child follow an instruction such as "Touch your right ear with your left hand." The child is first asked to identify the hands correctly, then the body parts. He is then asked to recognize the segmented quality of the longer instruction in carrying it out. That is, he may recognize that there are at least two parts into which the instruction may be divided. The issue is not whether or not he has a problem in following more complex commands. Rather he is offered a possible solution. As he uses the adaptive strategy of "segmentation" it is explicitly identified, and he is reinforced for having used it as well as for the results obtained.

In a similar fashion the child is either told or shown or moved through a second series of tasks. The objective now is to illustrate the value of using different modalities of input. For example, the child is told to "First draw a straight line, then a circle, then a straight line." If he is incorrect, he is then offered several options that may be used. He may ask the examiner to "segment" the instruction by saying it again more slowly, as had just been demonstrated on the right-left tasks. In addition, he may ask the examiner to show him or move him through the activity as well as tell him. Again the child is given recognition for defining a problem-solving strategy as well as for the results obtained.

The third set of tasks is designed to illustrate the aspect of competing stimuli. After a demonstration, the child is asked to connect circles randomly printed on a sheet of paper. After the behavior of connecting the circles is established, the child is then asked to connect circles on a second sheet containing competing geometric forms (lines, crosses and triangles). After a timed interval of connecting circles in the presence of the visual distractions, the examiner then names the competing figures as well for an equivalent length of time. Usually 10-second intervals are used. The child now is offered the option to connect circles on a sheet of paper identical to the one used for initial training where there are no competing figures on the sheet and the examiner remains silent. The number of circles connected during this third 10-second interval is compared to the performance during the previous intervals. It is generally higher than under the previous conditions. The child is given recognition for having requested the option of reducing distracting stimuli as a problem-solving strategy as well as for his increased rate of performance.

Discussion

This clinical model is an intrinsic part of a large-scale service program for children with developmental problems (16). The issue to be explored here is the implication of such an examination for a model of brain function. It has been suggested that the evaluation of a functioning organism must reflect those characteristics which make it an organism. One such aspect is the activity of the organism toward his or her maintenance and self-realization in relationship to the surrounding environment. Such a relationship is considered an interactive one. The examination model is explicitly designed to reflect this quality.

What the examiner does is determined by the needs of the child in terms of modifying the conditions in which the child carries out the prototype tasks. Moreover, the options are to a degree determined by the child. The particular options illustrated during the NDO are but a sample. They do offer an initial approach by which one may vary the stimuli provided in terms of time (segmentation across time), kind (input modality), and space (number of competing stimuli at any one time). As such, they offer specific problem-solving strategies that may be applied to other tasks of higher priority in the real life setting for the child. The number of strategies illustrated during any specific time period is limited by the constraints for effective communication in an information-processing framework.

It is not, however, the particular strategies or their number that are crucial. It is the recognition that one may explore strategies for solving problems that is being simulated. If one is to evaluate brain function in the area of development problems, the objective of such evaluation is different from the classic issues of clinical neurology. It must be to define options for enhanced development. It is the view of the organism as an adaptive system that is being modeled. The very process of examination may then be the context for such enhancement.

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The Case History of Monkey No. 109: First Experimentally Produced Vertical Gaze Palsy*

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One of Bender's major contributions to the understanding of neurologic problems is his proposal for the mechanisms involved in moving the eyes along the vertical plane (1). In short, the concept states that *bilateral* innervation is required to induce vertical ocular deviation, and that *bilateral* lesions are necessary to produce vertical gaze palsies. Such a conclusion derived from numerous examples of patients with neurologic disease and the results of experiments in monkeys. These two bases, clinical and experimental, have a common root in Bender's approach to neurology, i.e, the strict observation and the complete, most simple thought-economic description of phenomena with absolute disregard for explanations. Phenomenalism and the principle of parsimony are easily recognizable in this general way of acquiring scientific knowledge. Both were born from Machian positivism which is based on a gradual step-by-step adjustment of ideas to the facts from a strictly neutral position, purely observing and registering phenomena (2). This position in the philosophy of science originated as a strong reaction against verbal explanations which very commonly interfere with the accuracy of new observations. The more modern movement of phenomenology, which developed from the ideas of Edmund Husserl, has preserved these general principles (3). Husserl's motto was "To the Things" (*Zu den Sachen*) meaning a de novo examination of phenomena devoid of preconceived ideas in the attempt to describe them as faithfully as possible. Phenomenology had a great impact on psychology where the work of William James and later that of the Gestaltists had been developing along parallel lines.

The influence of Gestalt psychology in Bender's work has also been quite marked. He has given a prominent role to the background in considering the effect of any stimulus figure, and his discoveries of perceptual interactions have a definite Gestaltic flavor (4, 5). A perhaps more relevant feature to the main topic of this communication, which appears most notably in Bender's neurologic thinking, is the holistic concept of brain function masterfully discussed by his longtime friend, Kurt Goldstein (6). Bender's refusal to accept the existence of brain "centers" permeates from all his writings and teachings. A particular

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subject in this problem area was the negation of the traditional view that the superior colliculi were the "centers" for vertical gaze. It is tempting to speculate about the possibility that Bender may have been inspired by the work of William G. Spiller in drastically dismissing such role of the tectum. While holding the titles of Professor of Neuropathology and Associate Professor of Neurology at the University of Pennsylvania Medical School, Spiller became president of the American Neurological Association in 1905. Departing from the usual themes for such occasions, his presidential address dealt with an original piece of research. [This change was also evident in Bender's subject for his address as President-elect of the same Association 67 years later (7).] The title of Spiller's lecture was "The Importance in Clinical Diagnosis of Paralysis of Associated Movements of the Eyeballs (Blicklaehmung), Especially of Upward and Downward Associated Movements" (8). He stated: "All the pathological evidence that I have been able to obtain in cases of persistent palsy of associated upward or downward movement is indicative of a lesion near the aqueduct of Sylvius. It is extremely doubtful whether a lesion confined to the corpora quadrigemina and causing no pressure on the surrounding parts ever causes paralysis of associated ocular movements, and those who favor such a view have not produced proof of a supranuclear center in this part" (p. 424). And also that "it seems unnecessary to assume that a coordinating supranuclear center for upward or downward movements exists in the corpora quadrigemina or their vicinity" (p. 426). More recently, the myth of the superior colliculi as "centers" for vertical gaze was definitively dispelled in a detailed study of oculomotor function including spontaneous eye movements and evoked nystagmus in the monkey (9). Spiller occupied the chair of Neurology from 1915 until his retirement in 1932. He was therefore the man who taught Neurology to Bender while the latter was a medical student at the University of Pennsylvania. Bender has always been proud to acknowledge that he worked under Spiller, and to some extent many of Spiller's personality traits and methods relative to patients and other scientific matters, as described by some of his biographers (10), can be found also in Bender. Spiller, however, failed to recognize the crucial aspect given by the bilaterality of the lesion to produce disturbances in vertical gaze, despite the fact that his 3 cases with postmortem examination, out of a total of 9, appeared to have bilateral pontine tegmental lesions. The concept of bilaterality, indirectly suggested by others (11, 12), was extended by Bender to imply that the pathways for vertical gaze are the same as those for horizontal gaze acting simultaneously (1).

The main purpose of this contribution is to document the first experimental evidence that the pretectum of the monkey contains critical structures for movements in the vertical plane, particularly upward. In addition to being one of the bases which led Bender to postulate the concept, the detailed protocol available on this monkey will also serve to bring forward Bender's unrelenting enthusiasm for the observation of new phenomena during a most trying period of his life. Histologic reconstruction of the lesions as well as all examinations and procedures were performed by the authors, except for those made from 4/

17/56 to 6/29/56. Either Dr. H. P. Krieger or Dr. S. Shanzer recorded the observations during this lapse. To economize on space, the details of the admission note to the monkey colony and of repeated examinations with identical results are omitted.

Protocol of Monkey No. 109

3/19/56: Young macaque, weighing 3.5 Kg, neurologically normal both by examination and recording of optokinetic and vestibular nystagmus.

3/21/56: An attempt will be made to destroy the left superior colliculus by electrolysis through stereotaxically placed electrodes. The coordinates were selected according to the Horsley-Clarke atlas (13). Three electrodes were mounted on the left holder and carrier of the Johnson stereotaxic instrument, 2 mm apart, and placed in sterilizing solution overnight.

3/22/56 (11:00 A.M.): Aim of the operation: Twelve electrolytic lesions in the left superior colliculus without damaging the cerebral hemisphere. Sterile technique under sodium pentobarbital anesthesia 100 mg i.v. and 50 mg i.m. during the procedure. An ample left craniectomy was made from 1 cm behind the vertex to the occiput and from the midline to under the insertion of the temporal muscle. The animal was then mounted on the Johnson stereotaxic apparatus. Dura was opened and turned on the sinus. Surface of the brain was protected with cotton felt wetted with saline solution. The carrier with the electrodes set at A 0, A +2, and A +4 was fixed at L 1, between the hemisphere and the falx. The superior aspect of the corpus callosum was visualized. This was one point of reference with respect to the possibility of interanimal variation. The H coordinate coincided with that in the atlas. Lesions were made by passing a current of 5 mA during 45 seconds. An interval of at least 5 minutes was left between lesions. The observations which follow were made during each electrolysis and were witnessed by Dr. H. P. Krieger.

A 0 L 1 H +2.5 Twitching of the eyelids.

A +2 L 1 H +3.5 Twitching of the eyelids. Slow right conjugate horizontal deviation.

A 0 L 1 H +4 Twitching of the eyelids. Recurrent right conjugate horizontal deviation.

A +4 L 1 H +4.5 Vertical nystagmus downward for the entire duration of the electrolysis. The amplitude was slightly greater in O.D. This was seen also by Dr. P. Bergman.

The electrodes were then repositioned at L 2.5 without touching the cerebral hemisphere.

A 0 L 2.5 H +2.5 O.U. to midposition. After electrolysis, left conjugate deviation with downward vector.

A +2 L 2.5 H +3 Fixed in right conjugate horizontal position even with current off.

A +4 L 2.5 H +4 Hippus. Miosis. Quick rotatory movement down and to the right; then to left conjugate gaze. The animal vomited profusely and aspiration was immediately started. Nothing else was done in the next 20 minutes.

A 0 L 2.5 H +4 No reaction. O.U. fixed in midposition. After electrolysis, conjugate downward gaze.

A +2 L 2.5 H +4.5 O.U. to midposition and then right conjugate horizontal deviation. After lesion: downward conjugate gaze.

Electrodes were removed again, cleaned and reinserted at L 4. This time 2–3 mm of the supracallosal convolution were damaged by the two anterior electrodes.

A +2 L 4 H +2.5 Slight right conjugate gaze. After lesion: conjugate downward gaze.

A +2 L 4 H +4 No eye movements.

The electrodes were disinserted, the animal taken off the apparatus, and the wound closed in anatomical layers. Operation ended at 3:00 P.M.

3/22/56 (9:30 P.M.): Animal is fully alert. There is vertical nystagmus downward, particularly on unsuccessful attempts to look upward. Pupils are widely dilated and react sluggishly to light.

3/23/56: Monkey in good general condition. There is marked retraction of the eyelids. Upward gaze is definitely impaired. Conjugate horizontal and downward gaze are intact. Occasional vertical nystagmic jerks in a downward direction. Spontaneously, the eyes are usually in a downward and inward position. Animal follows a light in all directions but upward. Pupils are dilated but react to light better than yesterday.

3/34/56: Condition is essentially the same except that the pupils are less dilated. The reaction to light is still sluggish.

3/26/56: The essential features today are: 1) marked retraction of eyelids; 2) paralysis of upward gaze; 3) vertical nystagmus, quick component downward without passing the horizontal plane during the slow phase, present when the animal makes an effort to look upward; 4) animal looks preferentially to the left although it is able to look to the right; 5) pupils are equal, semidilated, and react sluggishly to light. Dr. Bender was informed about this monkey and said that the lesion must be in the region of the posterior commissure rather than in the superior colliculus.

3/28/56: Condition is essentially unchanged.

3/30/56: Visual fields are normal. Animal directs gaze to objects placed in all quadrants. To look upward, however, it hyperextends the head instead of moving the eyes. Eye movements are normal except for paralysis of upward gaze on following targets. In contrast, the Bell's phenomenon is present, upward and to the left. There is vertical nystagmus downward on downward gaze. At times this nystagmus is of the shimmering type. Pupils are semidilated and react sluggishly to light. Corneals are present. Facial and motor V normal. There is a slight weakness of the right extremities with diminished placing on that side. Hopping reaction is normal and equal on both sides.

Bárány test in the upright position: After 15 clockwise rotations, there were 15 sec of horizontal nystagmus to the left. After counterclockwise rotation, the nystagmus was to the right. The test was then made with the monkey lying on its left side. Clockwise: during rotation O.U. went upward beyond the horizontal plane; after rotation there were some nystagmic jerks upwards lasting only 2–3 seconds. Counterclockwise: during rotation O.U. moved to the extreme downward position; after rotation, there was a first tonic deviation upward and then a vertical nystagmus downward of higher amplitude and lasting over 30 sec. The test was repeated in the same position. Clockwise: after rotation, there was a tonic deviation downward followed by shimmering nystagmus, then a few jerks upward of low amplitude without passing the horizontal plane and lasting 10 sec. Counterclockwise: after rotation, tonic deviation upward and then nystagmus downward of high amplitude and long duration.

Caloric test with iced water in the upright position. Normal response on irrigation of A.D. and A.S. In the supine position: vertical nystagmus downward on irrigation of A.D. Horizontal nystagmus to the right on irrigation of A.S.

Optokinetic drum (recorded). Monkey tended to fall asleep, nevertheless a good optokinetic nystagmus (O.K.N.) and optokinetic after nystagmus (O.K.A.N.) could be obtained in both direction and at all speeds. There was an unsuccessful attempt to obtain a vertical O.K.N. by placing the animal in a lateral position.

4/2/56: Examiners: Dr. Bender and staff in Rm. G358 (on the 8th postoperative day of Dr. Bender). The outstanding feature is marked retraction of upper eyelids. Both pupils are semidilated, but react to light and constrict well with eyelid closure. The eyes are usually in a downward position. Convergence is excellent. There is no apparent nystagmus. Horizontal eye movements appear to be normal. Those to the right are not so frequent as those to the left. The eyeballs do move with eyelid closure (Bell's phenomenon) and this seems more apparent on the right side. It should be noted that the monkey is in a restraining box and cannot perform free movements with the rest of the body. This animal does not move the eyes upward even on following grapes.

4/4/56: Neurologic condition is unchanged. Retraction of eyelids is present. Pupils semidilated. No spontaneous nystagmus. Paresis of upward gaze is not as marked as in previous examination.

4/6/56: This monkey was seen by Prof. Heinrich Klüver during his visit to our laboratory. At this time he mentioned that the superior colliculi have something to do with eye movements and that we must consult Byrne's book *Studies on the Physiology of the Eye* which is a classic on this subject. He did not remember having seen a monkey like this before.

4/9/56: Eyelid retraction is still present. It is more marked when the animal is changed from the upright to a supine position. It looks preferentially downward and slightly inward. Horizontal movements are normal. Convergence is normal. Upward gaze has definitely improved. On trying to look upward, the eyes pass occasionally the horizontal plane. Vertical nystagmic jerks are present sometimes on looking upward. Pupils are equal, less dilated than in previous examinations, and react to light.

4/12/56 (2:00 P.M.): Condition has improved. Eyelid retraction is not as marked as before and the monkey looks upward on occasions. In an attempt to precipitate and increase the symptoms it was decided to give this monkey chlorpromazine, 12 mg i.m.

4/12/56 (5:30 P.M.): Neurologic examination failed to reveal any defect. Palpebral fissures were normal as well as the eye movements were normal including those in the vertical plane. Pupils normal.

4/12/56 (8:00 P.M.): Eye movements are still normal.

4/13/56: The "paradoxical" improvement caused by the drug disappeared completely. Monkey shows again eyelid retraction and paresis of upward gaze. Pupils are of normal size and react to light.

4/17/56: The future of this animal was discussed with Dr. H. P. Krieger. In view of the previous opinion of Dr. Bender to save this monkey for histologic reconstruction of the lesion responsible for this interesting syndrome, it was decided to continue the follow-up for another two months. Thence, Dr. Bender will be consulted again about the convenience of performing a contralateral lesion in this monkey.

4/25/56: Optokinetic drum (recorded). With the animal upright, the response is normal in both directions. With the monkey lying on either side, there is no nystagmus when drum moves from head to foot. There is good O.K.N. and O.K.A.N. when the drum moves from foot to head.

Bárány test upright: equal and normal in both directions. Right side down: during clockwise rotation eyes have tonic phase down with quick jerks up. It is impossible to judge with accuracy whether the eyes move beyond the horizontal plane during these upward jerks. After cessation of rotation, nystagmus downward. Moderate oscillations of eyelids. During counterclockwise rotation, eyes have tonic phase up with jerks down; after rotation, there are marked repeated retractions of the eyelids, upper greater than lower but only a few small jerks of upward eyeball nystagmus.

Caloric (cold) test gave normal responses with the animal in upright position.

Convergence is intact. There is paresis of pursuit movements upward. In the dark, there is relative paresis of upward gaze when a flashlight is suddenly turned on above the monkey's head. When this light is to the left, right, or below, the eyes dart quickly toward light most of the time. Bell's phenomenon is present. During sleep, the eyes could be seen in the midposition. Pupillary reactions to light and to what appears to be accommodation are normal. There are occasional nystagmoid jerks and short runs of nystagmus downward when the monkey looks down or obliquely down. There is mild spontaneous retraction of the upper eyelids.

4/30/56: Repeated all examinations of 4/25/56 with same results.

5/15/56: Bárány test in left and right lateral positions and optokinetic test gave same results as on 4/25/56.

6/6/56: Eyelid retraction is still present. There is weakness of upward gaze although some upward deviation is possible. There are very infrequent nystagmoid jerks down and occasionally to the left.

Optokinetic drum (recorded). Same as 4/25/56.

Bárány tests (upright): Same as 4/25/56. Left lateral position: after clockwise rotation, shimmerings of short duration in vertical plane. No real nystagmus. After counterclockwise rotation, shimmerings as before but in addition there are several jerks of nystagmus with a fast component downward.

6/8/56: Convergence intact at and below horizontal plane. Absent above this plane. The paresis of upward gaze is less than on 4/25/56 and somewhat less than on 5/15 but it is still definite. Reaction to suddenly exposed luminous targets shows defect in upward gaze, but it is improved as compared to 4/25/56. Bell's phenomenon as on 4/25/56. Upon voluntary oblique downward gaze to either side there are a few (1–4) nystagmoid jerks toward the midposition. These jerks are usually noted when the eyes move suddenly.

Bárány tests: Upright: normal when turned in either direction. Left side down: during clockwise rotation, O.U. with tonic deviation upward and jerks downward. After rotation: O.U. with few rapid, fine and short-lived jerks in upward direction. Marked repetitive upper eyelid retraction. During counterclockwise rotation: O.U. with tonic phase downward and quick upward passing slightly the midhorizontal plane. After rotation downward nystagmus with much less eyelid retraction.

Caloric tests (cold) A.D or A.S. simulation elicited horizontal nystagmus, normal and equal in each direction when tested with head erect. With the head back, nystagmus changed to downward rotatory, and with the animal upside-down the quick phase was straight toward the feet. In no position could straight upward or upward rotatory nystagmus be elicited.

Optokinetic drum (recorded). Same as 4/25/56.

6/13/56: There is still a paresis of upward gaze, the eyes hardly moving above the mid-horizontal plane. Some downward nystagmus when the animal looks suddenly down.

Bilateral stimultaneous cold caloric test. Monkey on its back; 10 cc cold water in both ears simultaneously. The eyes moved first downward and then there was a large jerk in the downward direction. In about 20 sec this changed to upward nystagmus with eyes moving well above the mid-horizontal plane. When the monkey was placed in the upright position, the upward nystagmus became more marked. When turned upside-down the nystagmus changed to a counterclockwise direction with tendency to right lateral gaze. Placing the monkey on its back again brought out the upward nystagmus without rotatory component. The test was repeated and the same results were found. These phenomena were witnessed by Drs. I. Wagman, R. Werman and C. Papatheodorou.

6/14/56: Paresis of upward gaze with occasional upward jerks. Slight eyelid retraction on changing the monkey from supine to upright position. Bell's phenomenon preserved. Occasional brief sustained downward jerks on downward gaze.

Bárány tests with monkey in left lateral position. During clockwise (10 turns) rotation, eyes deviated tonically upward. After rotation, vertical nystagmus upward plus marked eyelid nystagmus. Nystagmus lasted 51 sec. and it was of small amplitude and irregular frequency. During counterclockwise (10 turns) rotation, eyes tonically down. After rotation, 11 sec of well-defined downward nystagmus with much less eyelid retraction than after clockwise rotation.

Bilateral simultaneous cold caloric tests. When the eyes were in positions along the vertical meridian, there was usually a jerky nystagmus downward but occasionally nystagmus upward was also seen. There was marked eyelid nystagmus. When eyes were deviated away from the vertical meridian, nystagmus changed to an oblique or rotatory direction with a tendency of rapid component to assume the general direction of gaze. This rotatory component was at times distinctly upward. It was attempted to record this upward nystagmus but it seems that it was masked by the marked repeated retractions of the upper eyelid. The spontaneous runs of downward nystagmus were well recorded.

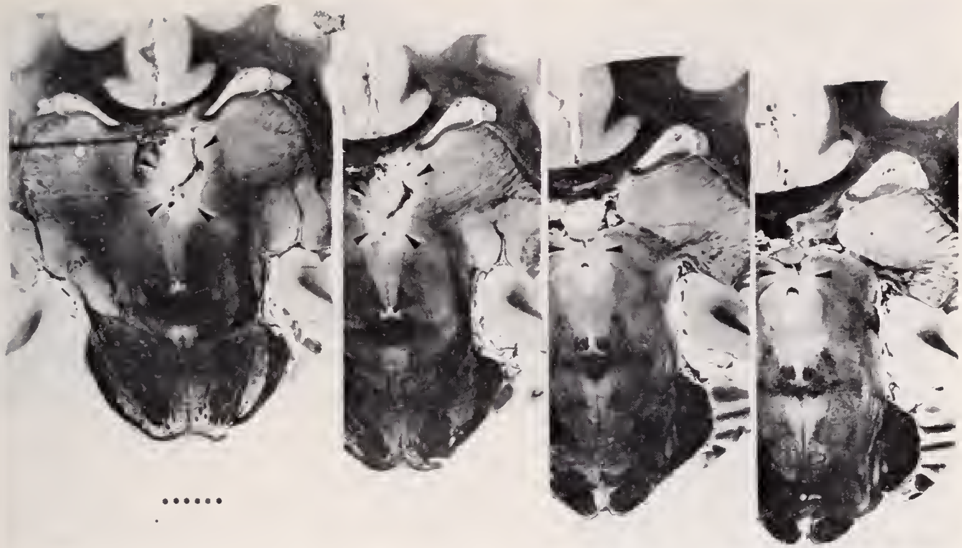

FIG. 1. Serial sections 1 mm apart. Myelin stain. Total scale: 5 mm. Lesions indicated by arrow points. Note that the damage is mostly on the left side but that it also crosses the midline to involve the right side. The posterior commissure is totally destroyed (two left sections).

6/22/56: Bilateral simultaneous cold caloric tests. Same results as 6/14/56.

6/29/56: Monkey was sacrificed. Brain was perfused with formalin and placed in same fixative.

11/3/56: Histologic examination of serial Nissl and myelin stained sections (Fig. 1) revealed that the principal extent of the damage was on the left side and included the habenular and pretectal regions, but that it also crossed the midline. The lesion destroyed the posterior commissure and involved bilaterally the nuclei interstitiales of the posterior commissure, the areas pretectalis and the habenular nuclei, with minimal involvement of the superior colliculi and central grey matter. In addition, there was damage to the nuclei pretectalis, Cajal and Darkschewitsch and to the thalamus on the left side.

Comments

In summary, the lesion that intended to destroy the left superior colliculus damaged instead the pretectum and habenular regions as a result of the faulty A plane coordinates given in the atlas. Indeed, a more recent atlas gives the colliculus coordinates posterior to the A +1 plane (14). The syndrome exhibited by this monkey was essentially the triad: 1) upward gaze palsy; 2) eyelid retraction; and 3) pupillary disturbances. In addition, there was spontaneous downward nystagmus, markedly defective vestibular nystagmus upward, and absent optokinetic nystagmus upward. These are the major components of the pretectal syndrome, recently described in full on the basis of a series of 15 animals which included Monkey No. 109 (15–17). Figure 2 (right) illustrates the facial features of such preparations, remarkably similar to one of Spiller's cases (Fig. 2, left).

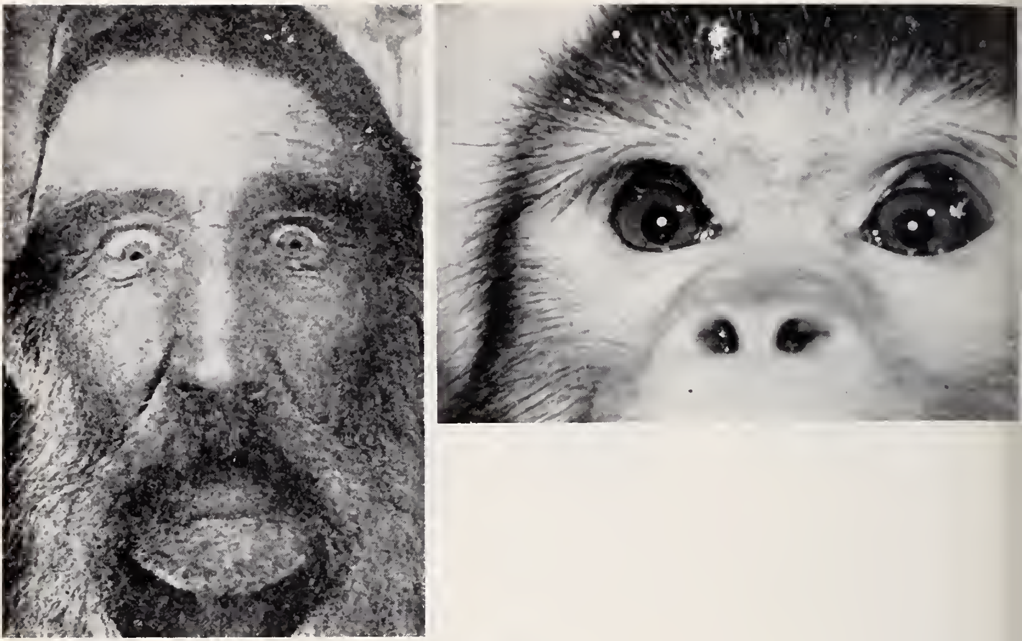

FIG. 2. On the left, the photograph of Spiller's Case No. 6 (8). On the right, monkey with section of posterior commissure. Note in both instances the retraction of the upper eyelids, the preference of gaze downward and in slight convergence, and the semidilation of the pupils.

There are several anecdotal comments which can be made on some entries of the preceding protocol. On 3/25/56 Bender underwent major abdominal surgery. The note of 3/26/56 refers to his opinion on Monkey No. 109 when we visited him on his first postoperative day. Notwithstanding the seriousness of his condition, Bender's excitement about the results was characteristic of his enthusiasm for new facts as revealed by pure observations. We started already with the plans for him to see this monkey as soon as possible. On 4/2/56, the animal was taken in a restraining box and wrapped in sheets to the Guggenheim Pavilion and slipped into room 358 without being noticed. Bender literally jumped off the bed and examined the animal with great joy. He was eating a bunch of grapes and used them to attract the animal's gaze. One at a time he passed a grape in front of the animal's field of vision and ended up by feeding it to the monkey. This was repeated many times after which he was convinced that the eyes could not be driven upward. In the middle of the examination, a resident placed as a look-out announced the proximity of the head nurse. The monkey was rapidly wrapped again and hidden under Bender's bed. Fortunately the nurse's visit was short and the animal did not vocalize while under the bed. Another alarm signal proved to be false by the appearance of Dr. M. Nathanson, who joined this hectic session. Bender finally dictated the note as transcribed.

During his visit to the laboratory on 4/6/56, Heinrich Klüver commented with delight about the long day and almost entire night he spent in San Diego during World War II discussing with Bender multiple subjects related to perception, hallucinations, and eye movements. This was the origin of a long-lasting mutual admiration. In fact, Klüver's classic review (18) occupies a salient place in Bender's personal collection of famous reprints.

On 4/17/56 arrangements were made with Dr. H. P. Krieger to follow up Monkey No. 109 because the authors returned to their country of origin, Argentina. They came back, however, 6 months later because conditions at the Faculty of Medicine of the University of Buenos Aires, prevented them from pursuing neurophysiologic investigations. Bender had fully recovered by this time and encouraged the authors to resume their work at Mount Sinai. During these 6 months examinations on Monkey No. 109 were performed by various members of the staff as noted.

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The Value of Handwriting in the Neurologic Examination

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Over the past four years at the suggestion of Dr. M. B. Bender, a study of the handwriting of patients admitted to the neurological service of The Mount Sinai Hospital has been conducted. In this paper, the method for obtaining and evaluating the handwriting specimen shall be presented. Emphasis, however, is placed on the value of the specimens in providing documentation of neurologic dysfunction and a chronicle of the patient's clinical course. Several observations shall be made on the comparison of specimens obtained from patients with aphasia and those with an organic mental syndrome.

The abnormal handwriting specimen is a clinical sign whose validity is incontestable as long as certain simple test conditions are met when the specimen is obtained. In this study, all patients were encouraged to assume the position most comfortable for writing, the lighting was made optimal, and eyeglasses were worn when indicated. Handwriting was tested in a hierarchical fashion. First the patient was asked to "Write anything." If the patient wrote his name, he was then asked to "Write anything but your name." The patient was next asked to write to dictation. Finally, the patient was asked to copy a sentence. Serial examinations were performed whenever possible. In addition, an attempt was made to obtain samples of a patient's handwriting prior to his admission to the hospital and preferably before the onset of symptoms.

As there is no standardized method for the evaluation of handwriting specimens, the following approach was used. The specimen was first evaluated as to how completely the patient performed the tasks required. That is, did he write on command, to dictation, and did he attempt to copy? The form of the specimen was then evaluated. This included how well the patient utilized the page, whether he wrote in a straight line, and how well he spaced his words. The morphology of the letters was then evaluated. The language content of the specimen is then studied for correct spelling, syntax, and meaning. Finally, the specimen is compared with previous ones, if available, and retained for comparison with future specimens.

Material obtained during the period of study from patients with cerebral dysfunction made the following observations possible. It was found that as a general principle there is a hierarchy of difficulty in handwriting. Writing on command is most difficult and copying the easiest. Writing to dictation is intermediate. In comparing the amount of written material obtained from patients with aphasia with that obtained from patients with an organic mental

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syndrome, it appears that the latter are much more prolific writers. There is a tendency for aphasics to leave the specimen incomplete and to have marked difficulty in spontaneous writing. Patients with an organic mental syndrome also leave incomplete specimens but less so than aphasics. A very interesting finding is that several patients with mild organic mental syndromes added material not solicited by the examiner, a phenomenon not observed in normal or aphasic patients (e.g., Figure 1B, final sentence).

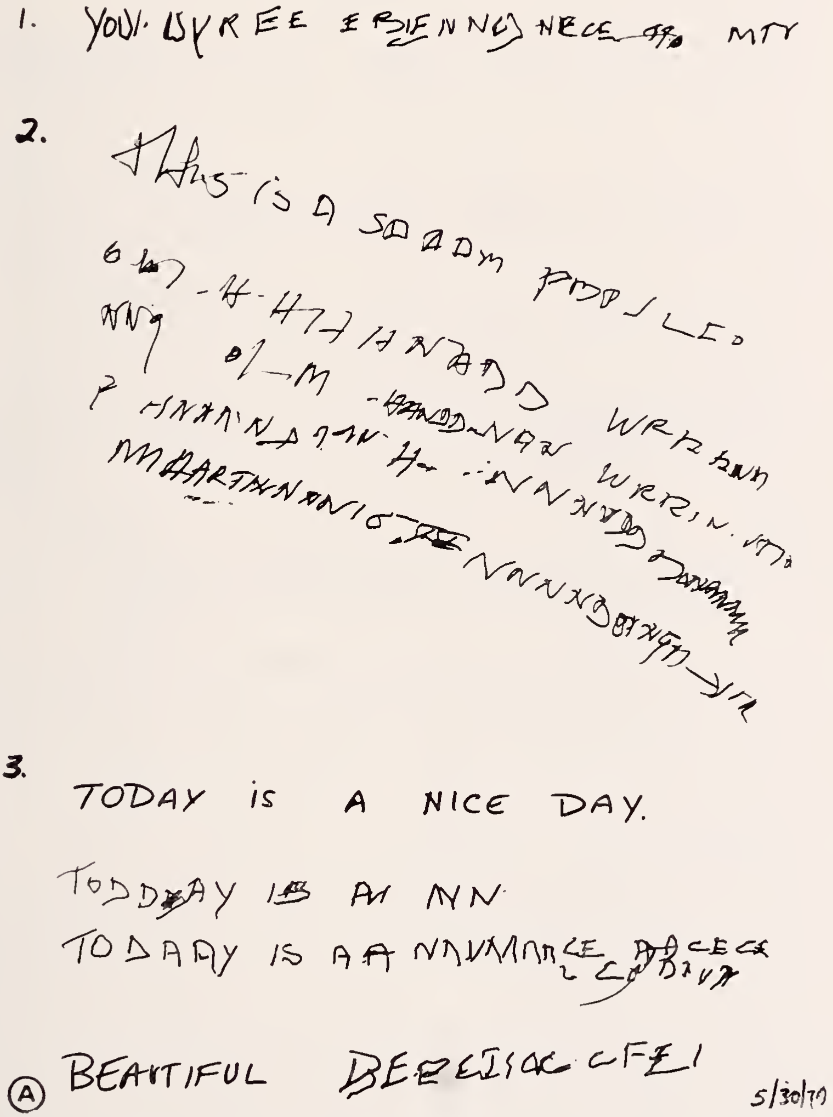
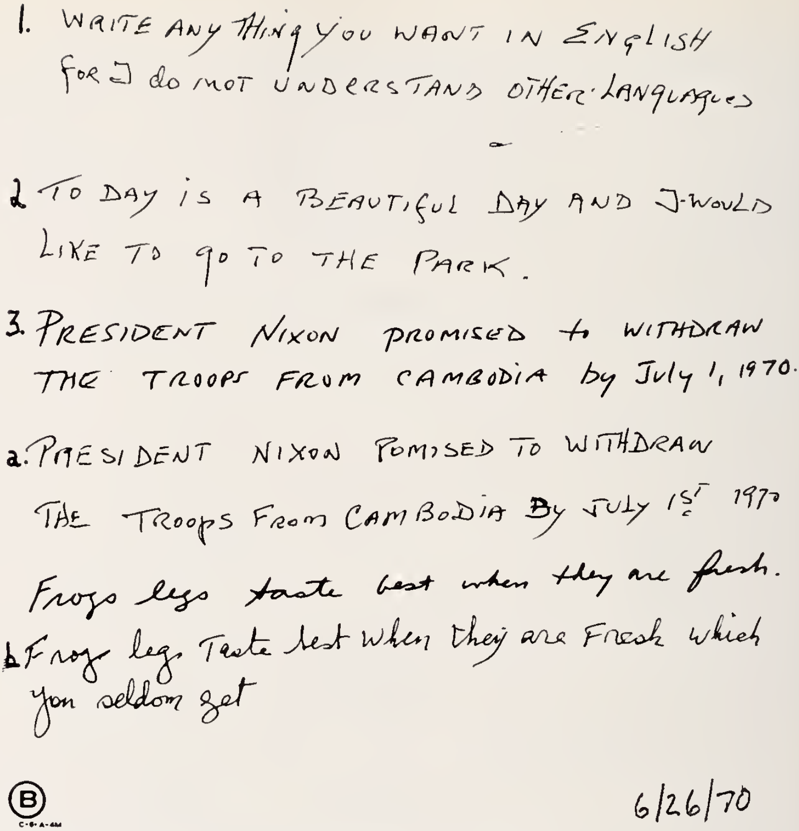


Fig. 1A. Specimen from M.D. early in hospitalization. 1. "You are being nice to me" written spontaneously. 2. "This is a sample of my handwriting" written to dictation. 3. "Today is a nice day" and "beautiful" copied.



1. WRITE ANY THING YOU WANT IN ENGLISH
FOR I do MOT UNDERSTAND OTHER·LANGUAGUES

2 TO DAY IS A BEAUTIFUL DAY AND I WOULD
LIKE TO GO TO THE PARK.

3. PRESIDENT NIXON PROMISED to WITHDRAW
THE TROOPS FROM CAMBODIA by July 1, 1970.

a. PRESIDENT NIXON POMISED TO WITHDRAW
THE TROOPS FROM CAMBODIA BY JULY 1ST 1970

Frogs legs taste best when they are fresh.

b. Frogs legs Taste best When they are Fresh which
you seldom get

Ⓑ

6/26/70

Fig. 1B. Specimen from M.D. later in hospitalization. 1. Spontaneous specimen. 2. Dictated specimen. 3. Copied specimen in printing (a) and script (b).

There is a suggestion that form is better preserved in specimens obtained from patients with aphasia. Again, samples from both groups overlap a great deal. Illegibility, micrographia, and macrographia were seen in both groups. Spelling errors were almost universal and included omissions, repetitions, and substitutions of letters or groups of letters. Perseveration of phrases and complete sentences was noted.

When serial specimens from the same patient were obtained, the value of this effort became readily apparent. A previous specimen either provided us with a normal baseline or helped to document earlier neurologic impairment. What is of special interest is the manner in which handwriting was found to reflect the deterioration or improvement in the mental status of patients with other evidence of aphasia or an organic mental syndrome. More specifically, handwriting was shown to be well correlated with the patient's speech and level of consciousness. In Figures 1 and 2, handwriting specimens are shown from two patients whose clinical course was documented with serial writing specimens. Patient M.D., whose specimens are shown in Figure 1, was a 59-year-old man with a reactive meningitis. The initial specimen was obtained when the patient

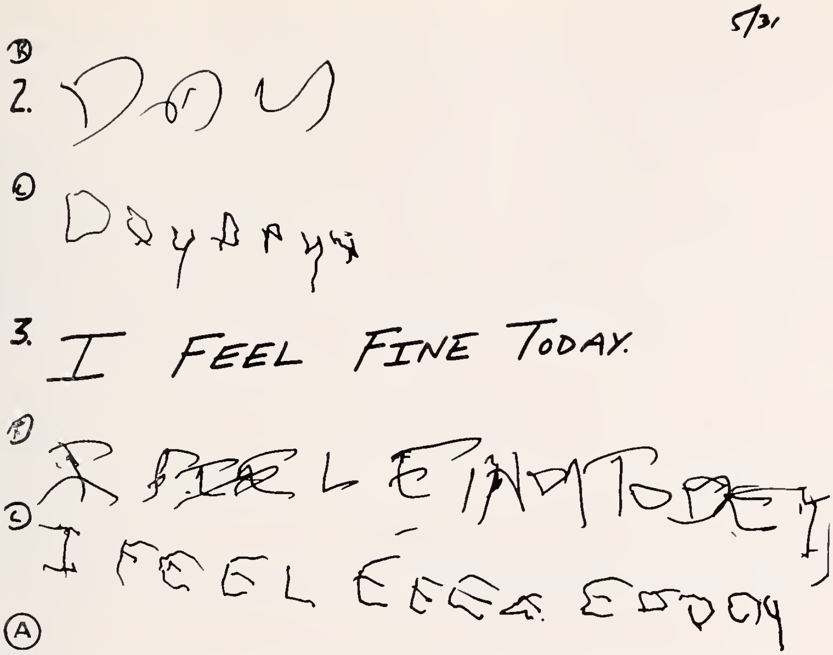


Fig. 2A. Specimen from J.W. early in hospitalization. Patient was unable to write spontaneously. 2. "Today is a nice day" written with the right (R) and left (L) hand respectively, to dictation. 3. "I feel fine today" copied with the right (R) and left (L) hand respectively.

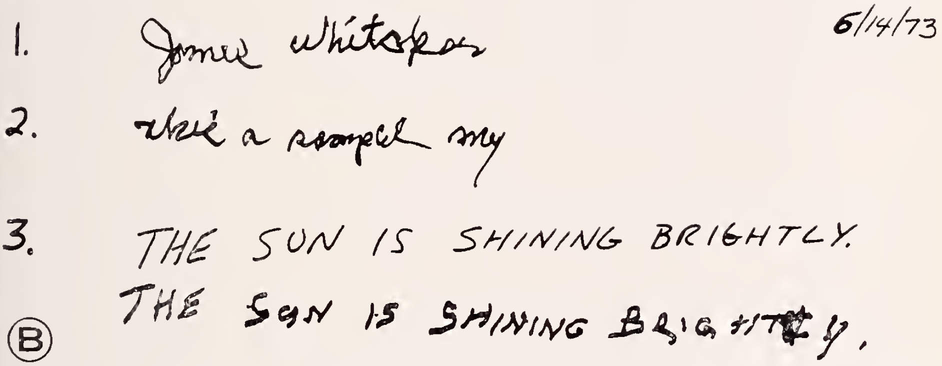


Fig. 2B. Specimen from J.W. later in hospitalization. 1. Spontaneously, patient only wrote his name. 2. "This is a sample of my handwriting" written to dictation. 3. "The sun is shining brightly" copied.

had a severe organic mental syndrome characterized by gross disorientation, poor memory, inability to spell or calculate, and a positive face/hand test. There were no focal findings. The second specimen was obtained several weeks later when the patient had a minimal organic mental syndrome characterized by difficulty in spelling reversals and memory testing. Patient J.W., whose specimens are shown in Figure 2, was a 25-year-old man admitted with a severe aphasia and right hemiparesis. The hemiparesis resolved rapidly in that he was able to manipulate the pen reasonably well when the initial specimen was obtained. He was found to have an intracerebral hematoma in the left temporal lobe. He improved on steroids as documented by his writing specimens.

From the above examples, it is evident that handwriting is a phenomenon which can be easily elicited and preserved without contamination from the examiner. Serial examinations provide a reliable and permanent record of the patient's clinical course. The high sensitivity of handwriting in reflecting cerebral dysfunction was evident in patient M.D. and has recently been discussed by others (1). The value of handwriting in providing a chronicle of the patient's clinical course seems well established. Further investigation is indicated in the study of handwriting in patients with cerebral dysfunction.

Acknowledgments

The author thanks Drs. M. B. Bender and F. Rizzo for permission to examine their patients.

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Chemotherapy of Experimental Brain Tumors: Preliminary Studies*

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Brain tumors are a form of neoplasia particularly resistant to effective clinical management. In addition to being situated in an area difficult to reach surgically, the tumors are sequestered from effective chemotherapy by the blood-brain barrier which may prevent drugs from ever reaching the tumor (for review, see reference 1). This barrier may also block activity of the host's own immune defenses against the tumor.

Studies in this area have been hampered by lack of a suitable animal model to study: Brain tumor induction may be traumatic, inducing much nonspecific damage; or the extent, location and type of the tumor may be very random and determinable only after the animal is sacrificed and carefully studied (2). These limitations make the interpretation of experimental results difficult.

Recently we have been studying chemotherapy of gliomas induced in hamsters by intracerebral inoculation of Rous sarcoma virus (3, 4). This virus has previously been shown to induce gliomas in rabbits (5), dogs (6), and hamsters (7).

These preliminary studies report 1) the optimal virus inoculum for tumor induction, as well as the frequency of tumor appearance; 2) the tolerances of newborn hamsters to administration of the anti-tumor drugs CCNU [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; NSC #79037] (8) and prednisone [pregna-1,4-diene-3,11,20-trione; NSC #10023] (9); and 3) the effect of the administration of these drugs upon subcutaneous astrocytomas and the virus-induced brain tumors.

Methods and Materials

Animals. Syrian golden hamsters or albino C-57 mice were utilized in these studies. Pregnant females were caged separately, the newborn animals receiving virus intracerebrally, usually within 24 hours after birth. Daily records were kept of animals' weight and general development.

Virus. The Schmidt-Rupin strain of Rous sarcoma virus was purchased from the Electronucleonics Company, Bethesda, Md. Preparations are provided titered in terms of

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tumor-inducing units (TIU), reverse transcriptase activity, and particle counts. Samples used contained 10^{11} TIU/ml. For use, virus was diluted in PBS (phosphate buffered saline, pH 7.2). The virus was inoculated intracerebrally, on the animal's right side, in a volume of 0.01 ml.

Drugs. The drugs used in these studies were CCNU (8) and prednisone (9). A solution of CCNU in ethanol and then suspended in PBS was administered intraperitoneally, once, at determined times. A suspension of prednisone made in ethanol and PBS was administered intraperitoneally, usually once every three days. Drug doses were delivered in 0.1 ml. volume.

Assay for the Presence of Glioma. The appearance of a tumor within the brain after virus inoculation was determined three ways: first, by gross inspection. Often the tumor was discernible as an extracerebral mass. The tumor frequently assumed a bilobulated shape, presumably growing through the small hole made by inoculation. These tumors also had one intracerebral lobe. Second, smaller tumors could be identified histologically by paraffin embedding and hematoxylin-eosin staining. And third, intraperitoneal injection of 1% trypan blue in PBS often selectively stained the tumor: The increased vascularity of the glioma caused the stain to accumulate more heavily here than in normal brain.

Induction of Subcutaneous Astrocytomas. To measure the effect of drug doses on neoplastic growth outside of the brain, hamster subcutaneous astrocytomas were studied. These tumors were induced by the implantation into newborn hamsters of 10^6 astrocytes transformed by SV-40 or polyoma viruses (10). These cells were maintained in tissue culture by techniques previously described (11–13). The presence of subcutaneous tumors was determined by gross inspection.

Results

Table I summarizes the incidence of tumor formation in hamsters receiving varying doses of Rous sarcoma virus intracerebrally. The percentage of animals having tumors increases as the virus inoculum increases. It was determined that inoculation of 10^9 TIU is the optimal dose for tumor induction. Figure 1 shows the appearance of a brain tumor within 3 weeks of virus inoculation, along with a normal animal. This proved to be a bilobulated tumor, growing inside and outside the brain case. Histologically these tumors are gliomas which show stages of astrocyte proliferation, and are permeated by many small blood vessels.

Table II shows the incidence of gliomas in newborn hamsters and mice receiving Rous sarcoma virus. The rate of successful tumor induction is approximately 80%.

TABLE I

Initiation of Hamster Gliomas by Rous Sarcoma Virus: Viral Dose Dependence

% Animals with Tumors	Inoculum Size in 0.1 ml (* TI Units)
0	10^3
0	10^5
2	10^6
15	10^7
80	10^9
81	10^{10}

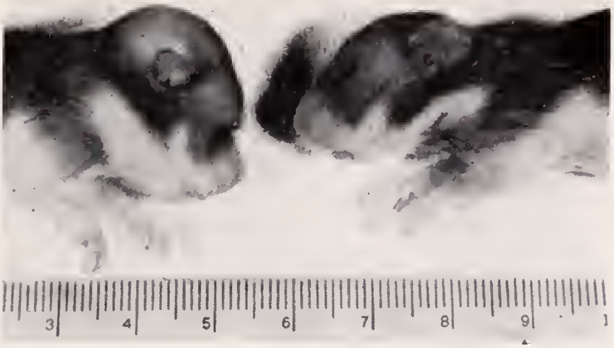

Fig. 1. Appearance of brain tumor within three weeks of intracerebral inoculation of Rous sarcoma virus (left). Upon dissection, tumor was bilobulated, one lobe being extracranial and the other within the brain. Histology of the tumor shows astrocytes in various stages of proliferation and infiltration of small blood vessels.

TABLE II

Incidence of Gliomas Induced by Rous Sarcoma Virus in Newborn Animals*

	Day after Virus Inoculation				
	0	30	60	90	120
Syrian Golden Hamsters	0/12	16/20†	18/22	15/19	ND
Mice . .	0/13	13/16	8/12	ND‡	ND

* Intracranial inoculation of 10^9 infectious units of Schmidt-Ruppin Rous Sarcoma Virus.

† As determined either by histology, trypan blue vital staining or visual inspection.

‡ ND = not done; experiment in progress.

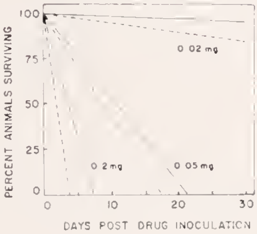

Fig. 2. Survival of animals receiving various doses of CCNU (– – –) and prednisone (——). Prednisone was administered every 3 days.

In order to determine the drug doses which permit optimal animal survival with anti-tumor effect, two experiments were carried out. In experiments summarized in Figure 2, the dosages of CCNU and prednisone were varied from 0.2 mg to 0.02 mg of each drug per 1-day-old animal. It can be seen that doses of either drug at 0.2 mg kill all animals within 7 days. Doses of 0.02 mg/animal of either drug permit over 80% of animals so treated to survive and this dose was used in experiments summarized in Figure 3.

To determine the anti-tumor effect of these drugs against peripheral solid

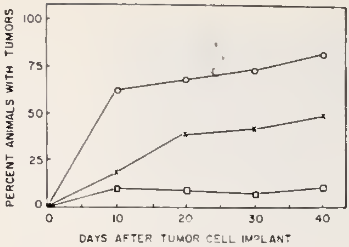

FIG. 3. Presence of subcutaneous tumors after implantation of 10^6 transformed astrocytes. ○——○ = no drug treatment ×——× = prednisone, 0.02 mg/animal given every 3 days; □——□ = CCNU, one dose given subcutaneously with cell implant, 0.02 mg/animal.

TABLE III

Appearance of RSV Tumors in Drug Treated† Animals*

	Day after Virus Inoculation	
	30	60
No Drug	12/15 (80)‖	20/23 (83)
CCNU‡	6/13 (47)	12/16 (75)
Prednisone§	5/9 (54)	11/18 (60)

* Intracerebral inoculation of 10^9 TI Units at birth.

† Drugs administered beginning 10 days after virus inoculation.

‡ 0.02 mg/animal administered once.

§ 0.02 mg/animal administered once every 4 days.

‖ Number in brackets is percent of animals seen to have tumors.

tumors (i.e., tumors not sequestered from drug therapy by the blood brain barrier), a population of hamsters bearing subcutaneous astrocytomas was produced. Figure 3 shows that with no drug therapy at 40 days after cell implantation, over 75% of the animals studied had tumors of varying size. Administration of CCNU and prednisone greatly reduces the percentage of tumor bearing animals. In these experiments, at least 100 animals were studied.

Table III shows percentages of animals bearing brain gliomas 30 and 60 days after inoculation with Rous sarcoma virus. The effects of CCNU and prednisone administration are shown. These preliminary experiments indicate that there is an anti-tumor effect of the drugs against the brain tumors which is not as pronounced as the effect noted against the subcutaneous tumors studied.

Further experiments are contemplated to repeat and confirm these observations.

Discussion

These preliminary experiments lend further support to the anti-brain tumor effectiveness of CCNU and prednisone. The Rous sarcoma induced gliomas in hamsters appear to be a suitable substrate against which to carry out evaluation of this effect. With the limits of extrapolation of animal studies to human systems (14 and 15) kept in mind, the results of these experiments may be directly relevant to the clinical management of brain tumors.

Because of the high degree of lipid solubility of CCNU (16) the drug appears to readily cross the blood-brain barrier and enter the brain. In these experiments the CCNU was administered intraperitoneally as an ethanol-PBS suspension. Increased anti-tumor activity of the drug may be brought about the suspension of the drug in highly lipophilic substances such as sesame oil (17), and the effectiveness of the drug delivered in this medium against the virally induced tumors should be measured.

In these experiments the optimal drug dose was determined to be 0.02 mg/25 g animal. This dose is about tenfold less than that previously reported for mice (17) but may reflect a species difference. The drug was administered at the same time as the initiation of the tumor, and further experiments showing the effects of drug administration after the onset of neurologic symptoms must be done.

The use of Rous sarcoma virus to induce brain tumors for chemotherapeutic studies appears to be promising. These gliomas may also provide material for studying the host's own immune response to brain tumors. Recent studies have indicated that the host's cell-mediated immune response may be artificially enhanced to combat neoplastic growth (18). The potential use of this against brain tumors remains to be elucidated.

Summary

Rous sarcoma virus was used to induce gliomas in newborn hamsters and preliminary anti-tumor chemotherapy experiments carried out. It was found that 1) the virus can be used to reproducibly cause brain tumors which lend themselves to chemotherapeutic studies, and 2) these tumors are inhibited by administration of prednisone and CCNU. Directions for future studies are discussed.

Acknowledgment

The authors gratefully acknowledge the expert technical assistance of Robert A. Michaels and Rochelle Cohen in these studies.

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Acute Occlusion of the Internal Carotid Artery without Anterior Collateral Circulation

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Attempts to relate the clinical manifestations of carotid occlusion to the angiographically demonstrable collateral circulation have generally been unsatisfactory. The majority of symptoms, their prognosis, and even the ultimate survival of the patient can usually not be predicted by the visualization or nonvisualization of collateral circulation or by its extent (1–5).

The author has collected data from 250 patients with angiographically proven internal carotid occlusions. Among this group, there were two in whom angiography failed to visualize the vessels of the afflicted cerebral hemispheres, despite separate injections of each common carotid artery. Neither the anterior communicating collateral from the patent carotid artery nor the external carotid to ophthalmic (or carotico-tympanic (6) or rete mirabile, etc.) collaterals from the diseased side was shown. Both patients died within 48 hours of admission and within 54 hours of the first onset of neurologic symptoms and are reported because of this rather rapid demise after the onset of symptoms of carotid occlusion, together with the lack of anterior collateral circulation. Unfortunately, brachial-vertebral angiograms were not performed in either patient and possible posterior collateral circulation** was not investigated.

Case 1. L.J. (M.S.H. #46368), a 51-year-old woman, was hospitalized on July 18, 1958 after being found unresponsive by her husband four hours prior to admission. There were no previous neurologic symptoms, and the patient had no significant past history.

The general physical examination was normal. The patient was globally aphasic, there was no speech, and she obeyed no commands. She appeared alert and followed the examiner with her eyes. Eye movements were full. She did not withdraw from threatening stimuli in the right field of vision, but did from the left. The right corneal response was absent and there was weakness of right facial movements. There were complete plegia of the right upper extremity and marked paresis of the right lower extremity. The right plantar sign was extensor and the deep tendon reflexes were increased on the right side. She reacted to pin prick bilaterally. Both carotids pulsated well; there was no bruit, and she did not cooperate well for ophthalmodynamometry.

Hemoglobin was 8.3 grams and there was slight elevation of the erythrocyte sedimentation rate. The remainder of the routine laboratory studies, including electrocardiographic and cerebrospinal fluid studies, were normal. The pineal gland was calcified and displaced 8 mm. to the right on skull roentgenograms.

The hospital course was characterized by some initial improvement; she could obey

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** Via the posterior communicating artery, posterior cerebral rete mirabile, posterior choroidal-anterior choroidal connections, carotidbasilar anastomosis, vertebral to occipital branch of the external carotid collateral, etc.

some simple commands from the left side of the bed. On the second hospital day, however, she became less responsive, and it was noted that her eyes deviated to the left and could not be made to cross the midline. Left carotid arteriography revealed complete occlusion of the internal carotid in the neck and no intracranial filling. Right carotid arteriography revealed the right anterior cerebral artery to be deviated to the right, a normal right middle cerebral artery and no filling of the arterial tree on the left. The shift of the anterior cerebral artery was thought to be the result of cerebral edema. The patient died 47 hours after admission and 51 hours from the onset of symptoms.

At autopsy the complete left carotid occlusion was confirmed and the clot extended into the left middle cerebral artery. There was massive encephalomalacia of the left cerebral hemisphere (Fig. 1) and fresh, midline, symmetrical hemorrhages in the mid-brain and pons.

Case 2. A.O. (M.S.H. #325659), a 68-year-old woman, was hospitalized on May 25, 1966 because of paresis of the right arm. There was a history of diabetes and of hypertension, both considered to be mild. At about 10 A.M. on the day of admission she noted a sudden onset of weakness in the right upper extremity, numbness of the right side of the face and difficulty with speech. These symptoms improved after two hours, and when seen in neurologic consultation at 1:30 P.M., there was only slight weakness of the right arm.

She was hospitalized at 4 P.M. Her blood pressure in both arms was 200/98. The remainder of the general physical examination was normal. Speech and mental status were felt to be intact. There was a slight right facial palsy, and a diminished right corneal response. She extinguished her right cheek, right hand and right foot with double simultaneous touch stimulation. There was a drift and some paresis of the right upper extremity. The reflexes and remainder of the neurologic examination were normal. The carotid pulsations were equal and there was no bruit. Ophthalmodynamometry revealed a pressure of over 150/20 in the right eye and about 120/10 in the left.

Routine laboratory studies were normal except for some glycosuria and ketonuria, elevated blood sugar, and left ventricular hypertrophy on electrocardiography. Spinal fluid and skull and chest roentgenograms were normal.

The day after admission the patient was noted to be semi-comatose, had a right hemiplegia, a dilated left pupil and gaze deviation to the left. Decerebrate posturing in response to painful stimuli subsequently appeared.

An electroencephalogram revealed bilateral abnormalities, much more marked on the left, and a left carotid arteriogram showed a complete occlusion of the internal carotid at its

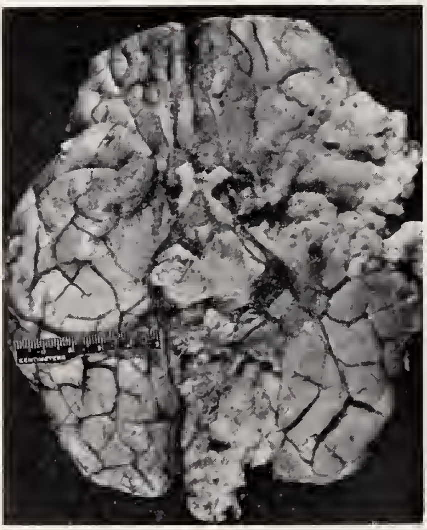

FIG. 1. (Case 1) There is extensive edema and malacia of the left cerebral hemisphere.

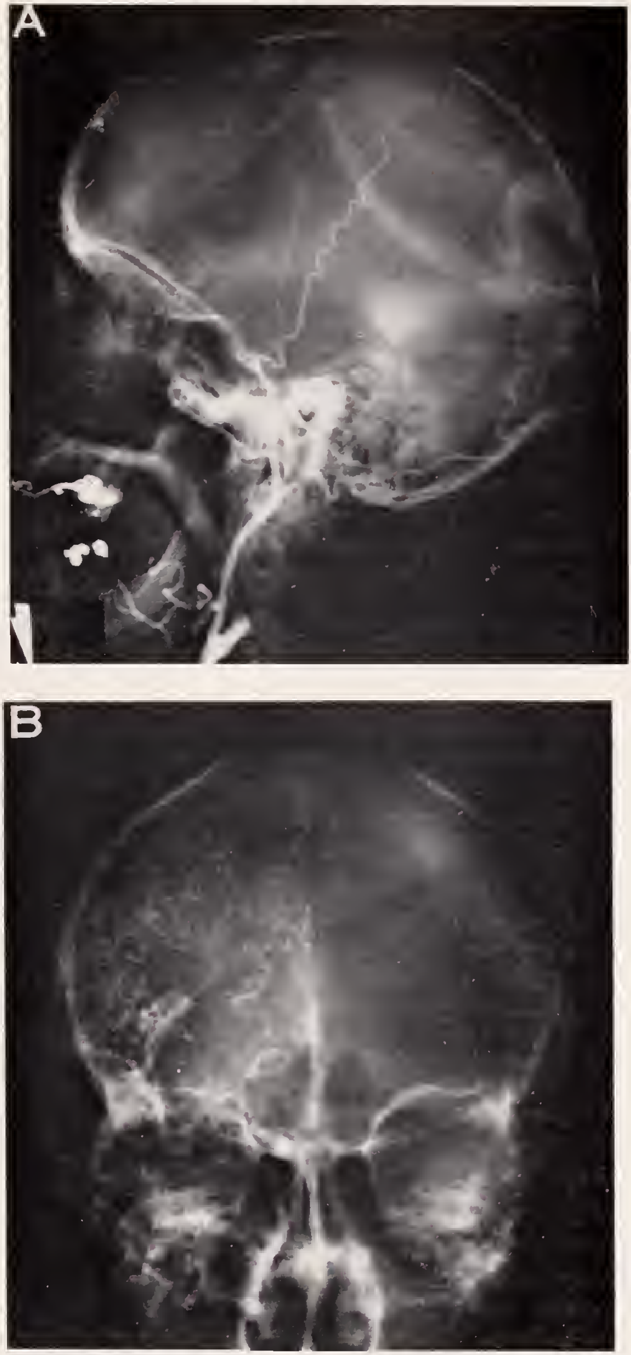

Fig. 2. (Case 2) (A) Lateral projection, left carotid arteriogram and (B) anterior posterior projection, right carotid angiogram, showing no visualized circulation in the left cerebral hemisphere.

origin, and no collateral by way of the external carotid. A right carotid arteriogram also failed to reveal any collateral to the left side (Fig. 2). She died 48 hours after admission and 54 hours after the onset of symptoms. Autopsy permission could not be obtained.

Discussion

These two patients have been reported because they represent the unusual occurrence of fairly rapid death after acute carotid occlusion without significant systemic disease or cerebral hemorrhage. Although Case 2 had some diabetic ketosis, this was treated promptly and did not seem to be causally related to her death. The hemorrhages noted in the brain stem of Case 1 were apparently terminal events, related to the (probably) increased intracranial pressure. It should be noted that both patients had brain stem involvement, patient 1 at autopsy. Patient 2 had a dilated left pupil and decerebrate movements, both of which were probably related to immediate causes of death.

It has been suggested that external carotid-ophthalmic collaterals are generally ineffective as collateral circulation (7, 8), and that they may appear when the anterior communicating circulation is inadequate (7). The two patients reported here did not have either of these or other anterior forms of collateral circulation; it is possible that their rapid deaths may be related to the relative avascularity of their involved hemispheres with resultant intracranial complications—e.g., edema, increasing pressure, etc., involving the brain stem.

Summary

Case reports of two patients who died within 54 hours after the onset of symptoms due to an occlusion of the internal carotid artery are presented. Bilateral carotid arteriography failed to demonstrate any anterior collateral circulation to their involved cerebral hemispheres. A possible cause and effect relationship is suggested.

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Responses and Interaction of Vestibular and Vegetative Systems*

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Introduction

The objective of the present report is to elucidate the vestibulo-vegetative phenomena especially related to respiration. Usually motion sickness cases respond to vestibular stimulation with accelerated respiration in addition to other changes—blood pressure, pulse rate, galvanic skin responses, etc. Deep breathing is usual with motion sickness. Vomiting, common with motion sickness, may be related to respiration (1).

Among many different approaches to motion sickness, the authors utilized electric pulse stimulation as an input and selected the peripheral nerve and the first station of the vestibular system as the site of stimulation. Several areas in the central nervous system and several peripheral nerves were stimulated in a similar fashion for comparison.

Method

Twenty healthy adult cats weighing 2.5–4 Kg were used for the study. Stimulating electrodes were stereotaxically implanted several days previously, in the vestibular nucleus, dorsal vagus nucleus, solitarius nucleus, and anterior and posterior hypothalamus in the cat. The electrodes consisted of three insulated 200 μ stainless steel wires glued together. At the tip of the wires, they were separated by 1 mm in the longitudinal direction. The points of stimulation were histologically identified. Operation and recordings were under general anesthesia with chloralose (30–40 mg/Kg) and urethane (100–150 mg/Kg), which were both administered through a venous catheter. On the day of studying responses, the electrodes to the vestibular nerve were implanted, through the labyrinth which was surgically destroyed, at the internal auditory canal.

The tips of electrodes were placed in the bony canal at the portion where the superior division of the vestibular nerve branches to the ampullae and the maculae. The labyrinths were bilaterally destroyed for equalizing the tonus and the electrodes were implanted usually on both sides. The other peripheral nerves such as the vagus, sympathetic, and sciatic nerves were bipolarly stimulated with stainless steel hook electrodes immersed in mineral oil. The interpolar distance of the electrode was about 1–2 mm. An arterial catheter for measuring blood pressure was introduced into the femoral artery. Spontaneous respiration of a tracheotomized cat was recorded by two rubber-band type resistors tied around the thorax and the abdomen. Electric pulses used for stimulation were square waves of 0.3–1.0

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msec duration and the frequency was 20–100 Hz. Pulses of 50 Hz and of 0.3 msec duration were most frequently used. Respiratory and circulatory responses were recorded on a polygraph.

Results

The vestibular nerve was stimulated with electric pulses of 0.3–0.5 msec duration. The responses appearing in respiration were: moderate suppression or augmentation for weak stimulation with less than 8 volts; irregular accelerated breathing (IABR) for intermediate (8–12 volts) or strong stimulation (more than 12 volts) and usually complete inhibition for strong stimulations (Fig. 1 A–D). The latency of responses varied a great deal. Irregular accelerated breathing remained the same after bilateral vagotomy performed at the level of the larynx. Responses in circulation from vestibular nerve and other stimulation were reported in the other paper (2).

Stimulation of the intact vagus nerve at the mid-cervical level introduced respiratory changes in addition to intensive circulatory disturbances. After bilateral vagotomy, the central cut end of the vagus on one side was stimulated, and sometimes highly accelerated respiration was seen with vestibular nerve stimulation (Fig. 1C), or, more frequently, strong and slow coughing-like respiration appeared (Fig. 2A). Coughing movement then was much stronger with the abdomen than with the thorax, and blood pressure was slightly depressed during stimulation. Blood pressure showed no depression, however, when coughing was intensive. When the peripheral cut end of the vagus was stimulated, more striking effects appeared in circulation: bradycardia and hypotension (Fig. 2B). With this stimulation, abdominal respiration was usually augmented in depth.

The vestibular nucleus, solitarius nucleus, anterior and posterior hypothalamus, and some other areas in the central nervous system were stimulated for comparison. Stimulation of the vestibular nucleus area frequently resulted in augmentation in amplitude and frequency of respiration during and/or after stimulation (Fig. 3A). Vestibular nucleus stimulation sometimes resulted in regulation of the rhythm and amplitude of respiration which lasted for a while after stimulation. Poststimulatory augmentation of respiration was occasionally marked, and the body motion then was more like retching, considering the tongue and body movements, increased salivation, etc. Stimulation of the posterior hypothalamus frequently caused respiratory changes which were usually facilitatory (Fig.

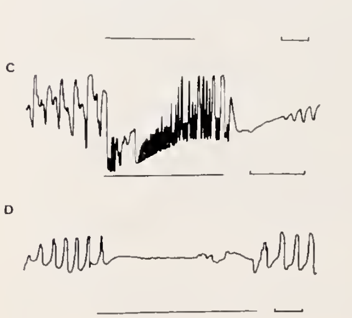
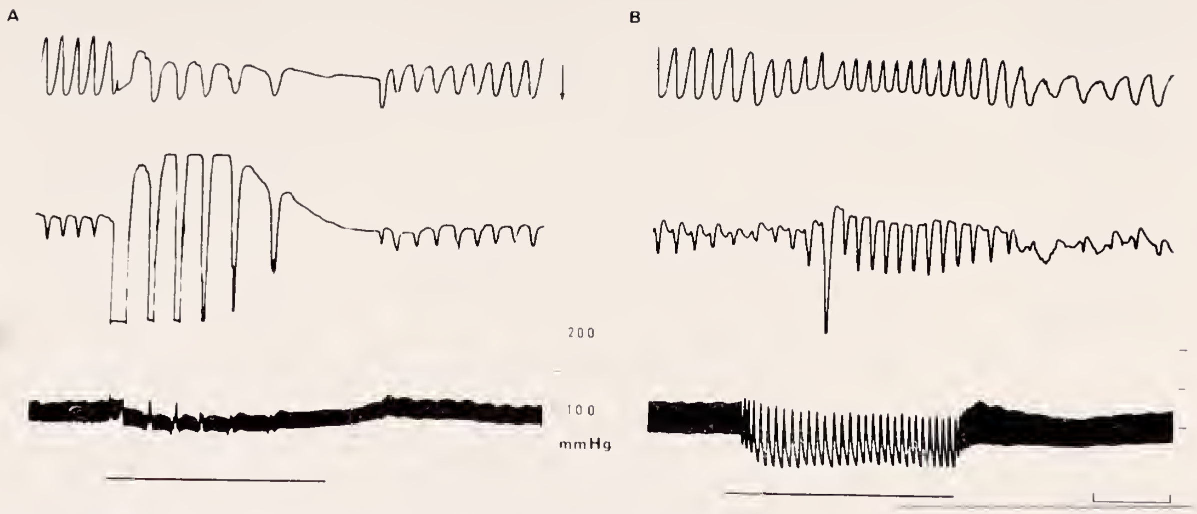


Fig. 1. Responses in abdominal respiration from stimulation of the superior division of the vestibular nerve in the internal auditory canal. The underlines in each of the recordings indicate the period of vestibular nerve stimulation. The respiration was spontaneous under general anesthesia with chloralose-urethane. The downward arrow indicates inspiration. The voltages of pulses for stimulation were changed: 8, 10, 12, 13 volts in A, B, C and D, respectively. Chart speed calibration is 10 sec.



Figs. 2A and 2B. Respiratory and circulatory reactions from ascending and descending stimulation of the vagus nerve. The vagus nerve was bilaterally cut and stimulated on one side with electric pulses at the central cut end in A and at the peripheral cut end in B. The top recordings in A and B are recordings of thoracic respiration, the middle, abdominal respiration and the bottom, blood pressure respectively.

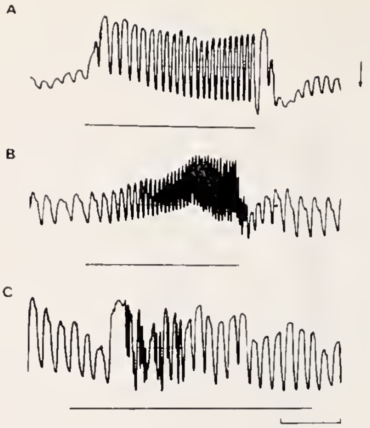


FIG. 3. Abdominal respiration from electric stimulation of localized areas in the central nervous system. The vestibular nucleus area (A), the posterior hypothalamus (B), and the solitarius nucleus area (C) were stimulated with 8 volts.

3B). The figure showed an example in which respiration rate gradually increased close to 100/min. This type of respiration is called polypnea (3). Stimulation of the solitarius nucleus area in most instances caused retching (Fig. 3C). The induced respiratory recording appeared irregular compared with the other two.

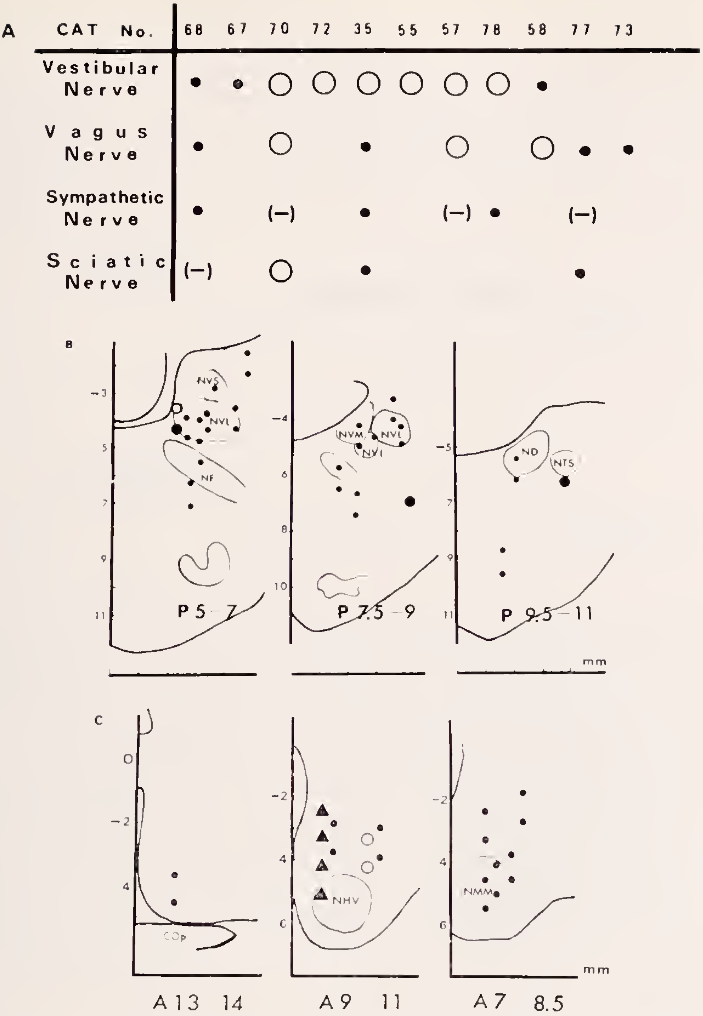
Electric pulse stimulation of the vestibular and the vagus nerves frequently induced brisk and fast respiration or IABR. The sciatic nerve was stimulated for comparison and this could cause IABR also, but it was not always successful (Fig. 4A). The cervical sympathetic nerve, however, could not induce IABR; but IABR could be induced in the relatively lightly anesthetized cat. Our experience with the series of animals is shown in Fig. 4 A–C. The intensity of stimulation applied in the series shown in Fig. 4 was restricted to train stimulations of square pulses of 6–10 volts, 50 Hz, and of 0.3 msec duraton. The sketch of the brain stem in Figure 4B showed correlation of the sites of stimulation and the responses induced. Any of the areas stimulated induced some responses which were either augmentation or suppression. Among these, there were the sites which responded by IABR or retching. Irregular accelerated breathing was induced as shown from the spot near the vestibular nucleus. Retching was from the areas near the vestibular and the solitarius areas.

Respiratory responses from the hypothalamus were, as illustrated in Fig. 4C, facilitatory or inhibitory; IABR appeared from the posterior hypothalamus. Polypnea with the frequency more than 100/min appeared from the medial portion of the posterior hypothalamus. The polypnea of a similar type was never seen from peripheral nerves.

When there were two different inputs, some interaction of repsonses between the vestibular and extravestibular systems was demonstrated (Fig. 5A).

The first input to the solitarius area induced regular respiration with larger amplitude and higher frequency than spontaneous respiration. The second input to the vestibular nerve appeared to have induced IABR motion superimposed on the respiration from the solitarius area. After vestibular nerve stimulation, respiration became less frequent and the behavior of the animal was more like retching. Summarizing the above, it appeared that solitarius and vestibular responses were just summated during stimulation, but that vestibular stimulation induced after-effects on the responses from single solitarius area stimulation.

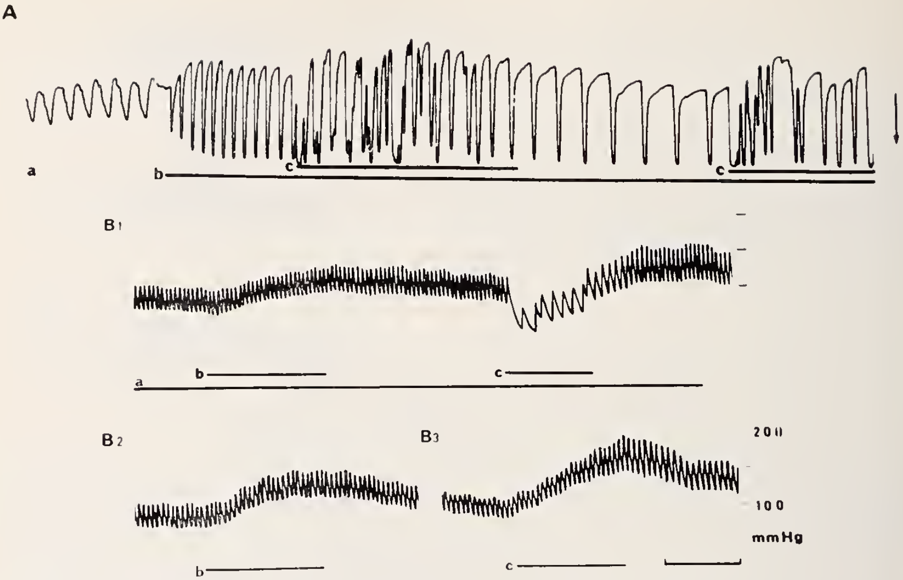
As seen in Fig. $5B_1$, blood pressure showed an elevation to combined sciatic nerve and dorsal vagus nucleus area stimulation, while, depression with bradycardia to combined vestibular nerve and dorsal vagus nucleus area stimulation. Sciatic nerve stimulation alone



CAT No.	68	67	70	72	35	55	57	78	58	77	73
Vestibular Nerve	•	•	○	○	○	○	○	○	•		
Vagus Nerve	•		○		•		○		○	•	•
Sympathetic Nerve	•		(–)		•		(–)	•		(–)	
Sciatic Nerve	(–)		○		•					•	

FIGS. 4A, 4B, AND 4C. Incidence of abdominal respiratory reactions from peripheral and central stimulations. A: the vestibular, cervical vagus, cervical sympathetic and sciatic nerves were stimulated with electric pulses of 50 Hz, 0.3 msec duration and with 8 volts. Numbers identify the cats used. ○ indicates irregular accelerated breathing (IABR), •, other positive respiratory responses with no IABR, and (—), no respiratory responses. B: respiratory reactions were plotted on the spot of stimulation in the brain stem at the levels indicated by P 5–7, P 7.5–9 and P 9.5–11. C: reactions were plotted in the hypothalamus at the levels of A 13–14, A 9–11, and A 7–8.5. ● indicates retching, ▲, polypnea above 100/min., and •, other respiratory responses. NVS, NVM, NVL and NVI are the superior, medial, lateral and inferior vestibular nuclei respectively. ND: dorsal vagus nucleus. NTS: tractus solitarius nucleus. NF: facial nerve. COp: optic chiasm. NHV: ventromedial nucleus of hypothalamus. NMM: medial mammillary nucleus.

or vestibular nerve stimulation alone both induced slight elevation of blood pressure with no bradycardia (Fig. $5B_2$, B_3). Stimulation of dorsal vagus nucleus area alone could induce hypotension and bradycardia, a similar response to the vestibular stimulation plus dorsal vagus nucleus area stimulation, with much higher stimulus intensity, however.



FIGS. 5A AND $5B_{1-3}$. Interactions between vestibular and the vegetative system. A: *a* indicates spontaneous abdominal respiration. In *b*, the solitarius area was stimulated with 6.5 volts. This induced frequent, high-amplitude curves in abdominal respiration. When vestibular nerve stimulation with 13.5 volts were added on this in *c*, irregularity in respiration was superimposed. Towards the end of the combined stimulation and, especially after vestibular nerve stimulation was discontinued, regular retching movements appeared with less frequency than those seen before superimposing vestibular stimulation.

In B_1, the area near the dorsal nucleus of the vagus was stimulated with weak stimulus of 2 volts as indicated by a long horizontal underline (*a*). The recording is of arterial blood pressure. Respiration was artificial. During stimulation to the dorsal vagus nucleus, stimulations to the sciatic nerve with 8 volts (*b*) and to the vestibular nerve with 12 volts (*c*) were separately superimposed. When sciatic nerve stimulation was added (B_1-*b*) an elevation of blood pressure was induced without changes in pulse rate, similar to the responses from sciatic nerve stimulation alone (B_2-*b*). When vestibular nerve stimulation was added (B_1-*c*) to stimulation of dorsal vagus nucleus area, marked hypotension with bradycardia were induced instead of blood pressure increase. Vestibular nerve stimulation alone (B_3-*c*) induced neither bradycardia nor hypotension as seen in (B_1-*c*). The dorsal vagus nucleus area alone induced hypotension with bradycardia similar to those in (B_1-*c*) but with much higher stimulus intensity.

Comments

The authors applied electrical pulses to two different areas of the vestibular system: one, the first neuron close to the labyrinth, and the other, the vestibular nucleus. The upper portion of the vestibular nerve was stimulated with bipolar electrodes in the bony canal where the utricular, anterior and lateral ampullary nerves bifurcate. Current spread can exist with higher voltages to the cochlear, facial, and intermedius nerves, although the majority of stimulus can be restricted in the upper portion of the vestibular nerve. Single ampullary nerve stimulation usually resulted in increased respiration. Since the electrodes used in this experiment, however, were much finer than those used in the present study, the stimulus intensity could not be then strong enough for inducing respiratory reactions similar to those in this report.

The vestibular nucleus was stimulated bipolarly with two tips separated by 1mm. Current spread, however, then should be considered to the adjoining structures.

The vestibular nucleus is close to the vegetative areas in the medulla: the areas responsible for salivation (5), vasomotor responses (6), spasmodic respiration (7), and so on. The lateral part of the reticular formation at this level includes many respiratory neurons according to a previous report (8). From the anatomy, and from the present technique of stimulation, electric current spread to those adjoining vegetative areas from the electrode at the vestibular nucleus is unavoidable. This may be true with other areas especially if the target is small, such as the solitarius nucleus. Marked vegetative responses from vestibular inputs in motion sickness may be a natural sequence on the anatomic basis. Fiber connections between the vestibular area and the lateral reticular formation at the level of the vestibular nuclei were electrophysiologically demonstrated (9).

For anesthesia, chloralose and urethane were intravenously administered. The anesthesia with these drugs is believed to be one of the best for the study of the vegetative system. The animal was thus kept anesthetized but not so deeply to inhibit respiratory responses.

Both ascending vagus and vestibular nerve stimuli usually induced IABR. These two different inputs may converge in the above-mentioned vegetative area near the vestibular nuclei which includes the solitarius nucleus. The solitarius nucleus is reportedly considered to be related to coughing (10). In connection with this, Fig. 5A demonstrated an interesting phenomenon. Vestibular nerve stimulation induced IABR and this was superimposed on hyperpnea which was induced from the solitarius nucleus area. Hyperpnea was more frequent before vestibular stimulation was added, and hyperpnea was less frequent and the animal behavior was more like retching after vestibular stimulation. This may indicate the existence of interaction between the vestibular and the solitarius area.

Retching in the cat may be a kind of respiration and may be related to vomiting. From his work with vagotomy and cord section at the cervicothoracic junction, Kimura concluded that retching was a specific type of respiration (11). The study done by Tang and Gernandt (12), which demonstrated increase of firing in the phrenic aand the recurrent laryngeal nerves from vestibular stimulation, may be related with coughing, retching, and IABR. Retching may be closely related to coughing or sneezing which were induced from the dorso-lateral portion of the medullary reticular formation (13). Vomiting was also induced from the similar area, the lateral reticular formation near the solitarius nucleus.

The present report also showed a phenomenon on blood pressure and pulse rate changes in Figure $5B_{1\text{-}3}$. According to this, vestibular inputs could induce "vagus responses" when the vicinity dorsal nucleus of the vagus was simultaneously stimulated. As shown in the figure, the sciatic and vestibular nerves by themselves induced similar responses of slight elevation of blood pressure. The stimuli to the dorsal vagus nucleus area could induce pronounced "vagus responses" with strong stimuli. Weak stimuli to the dorsal vagus nucleus area, which induced no responses, resulted in "vagus responses" when the vestibular inputs were added but not the sciatic inputs. This may indicate that the vestibular inputs may induce vagus responses with the aid of subliminal stimulation to a particular area such as the dorsal vagus nucleus area. This may explain the mechanism of motion sickness which is accelerated by extravestibular factors.

Conclusion

The input to the superior division of the vestibular nerve and the inputs to the solitarius nucleus area and also to the dorsal vagus nucleus area showed an interaction of respiratory and circulatory responses.

Summary

Several different types of respiration were induced by stimulation of peripheral nerves and central nervous systems, namely, suppression or augmentation of frequency and amplitude of different degrees. From observation of the animal behavior, facilitatory respiratory responses were similar to coughing, retching, polypnea, and so on. Vestibular nerve stimulation frequently induced irregular accelerated breathing (IABR), and vestibular nucleus stimulation induced varied respiratory responses, in addition to IABR and retching, and frequently rendered regulating effects on respiration. Ascending and descending vagus nerve stimulation at the mid-cervical level induced coughing or accelerated breathing and deep breathing respectively. Responses were different between abdominal and thoracic respirations. Stimulation of solitarius nucleus area usually induced retching. Combined stimulation of vestibular nerve and solitarius area resulted in interaction of responses different from those induced by either of the single stimulations. The interaction of responses from stimulation to these two systems were compared to the interaction of responses in circulation between vestibular nerve and dorsal vagus nucleus area.

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The Contribution of Morris B. Bender to the Study of the Oculomotor System

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The richness and diversity of disturbances in ocular motility had already attracted the attention of clinicians during the early classical period of neurology in the 19th century. Experimental investigations of this system were pioneered by Flourens (1824) and Cyon (1878) (1, 2). The first electric stimulations of the cerebral cortex made in the fundamental study of Fritsch and Hitzig in 1870 (3) elicited conjugate eye movements to the contralateral side. Research on eye movement mechanisms related to labyrinthine function, started with the now largely forgotten experiments of Högyes (4), who utilized mechanical recording methods, and transections and ablations. It is obviously impossible even to indicate the major lines of research, let alone to try to sum up the established facts concerning the still controversial neural apparatus for ocular motility. Fortunately, this is not the task of this paper's writer, who merely wants to appraise the position of Morris B. Bender in the recent spectacular development of this field of neurology.

There is hardly an issue in neuro-ophthalmology to which Bender has not made significant clinical or experimental contributions. However, I can visualize him primarily as standing on the dividing line: concluding the classical period of clinical and experimental studies and clearing the way for new approaches, particularly using systems theory, which belongs largely to the future. The careful and systematic studies of Morris B. Bender with various coworkers have disposed of a number of old beliefs that, in spite of contrary observations in subprimates, have been carried along notably in the clinical literature. The most remarkable feature of the Bender school is the convergent multilateral approach which combines clinical observations, precise recording techniques, systematic comparison of a sufficiently large number of cases (both clinical and experimental), meticulous exploration with electrical stimulation over widespread areas of the brain under stable conditions, and impeccable operating procedures with resultant well-documented lesions as verified by thorough anatomic study of all cases.

The fundamental general conclusion of these studies was that oculomotor effects, both as a consequence of stimulation, ablations or lesions, can be elicited from much larger areas than assumed originally. This appears to be true for cortical (5, 6) and brainstem stimulations and/or lesions (7). Although clinical and experimental evidence was available earlier, it was Bender (8) who put the bits and pieces together to firmly establish that, somewhere at the level of the oculomotor and trochlear nerve nuclei, there is a general decussation of pathways mediating eye movements. It was also Bender who rated this crossing as equal with and within the framework of the other major decussations of neural pathways.

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Being primarily a clinical neurologist, Bender's interest was understandably attracted to the anatomic and functional bases of paralyses of gaze. Immediately following his remarkable paper on functional representation in the oculomotor and trochlear nuclei (9) he proceeded to make the crucial observation for the understanding of the pathways for horizontal gaze (10). The latter study led to the conclusion that: "The ascending fibers in the median longitudinal fasciculus probably represent the terminal portion of the corticobulbar pathway for horizontal conjugate movements of the eyes." It is interesting now for the writer, who has worked very much along the same lines both in experimental techniques and paths of reasoning (11), to compare after 30 years the similarities and differences in observations and views. In spite of basic agreements in the parallel studies, I do not hesitate to admit that Bender's findings in this crucial matter were greatly superior to my own. (I might console myself with the thought that this is the advantage of the clinical neurologist over the neuroanatomist). Fortunately, for both the basic and clinical neurosciences, he stuck tenaciously to this topic and extended his stimulation and lesion experiments to wide areas of the brainstem (7). More recently, the continuation of these studies led to the anatomic identification of the pontine mechanism for horizontal gaze in the paramedian zone of the pontine reticular formation (12). These observations may finally dispose of the old belief of a "center for horizontal gaze" in the immediate vicinity of the abducens nucleus.

A common characteristic of the whole Bender school is its critical attitude toward the traditional concept of attributing certain more or less well-defined functions to discrete "centers" of the CNS. In most cases this is well justified, particularly with many old assumptions in clinical neurology being carried over from textbook to textbook. Moreover, recent discoveries with all their sophisticated techniques and views have not offered a better or more useful explanation for well-known clinical syndromes. A remarkable feat in this line was the conclusive disproval of the old idea that the superior colliculi play some essential role in vertical gaze movements. A major direct participation of the superior colliculi in oculomotor mechanisms of the monkey was ruled out in Bender's laboratory by the very careful studies of the Pasiks (13). These investigations were eventually developed further and led to the definition of the pretectal syndrome in monkeys (14, 15). Undoubtedly, the concept of the pretectal syndrome as outlined in these papers is probably the best that can be done at present, particularly with respect to offering useful guidelines for diagnostic purposes. However, in spite of my great admiration for this work, perhaps as an "old hand" at this game, I might be permitted to be slightly critical. I might point out that negation of the admittedly naive old "center" concepts should not go so far as to forget the necessity of an immediate premotor neuron system, however complex, that transmits oculomotor impulses to the motor neurons themselves.

Apart, perhaps, from extremely cumbersome studies on synaptic delays with intracellular techniques as used by the group of M. Ito (16) in Japan, there is no other means at our disposal to answer these questions than the study of terminal degeneration of premotor pathways in the oculomotor nuclei. Most of my own work (11, 17, 18) was conducted on cats during the pre-Nauta period, before suppression of the staining of normal terminal structures became possible in neurofibrillar methods. Consequently, tracing of degeneration was much more difficult than today and certainly needs to be reinvestigated in the monkey with improved histologic and electron-microscopic degeneration techniques. However, the basic synaptologic information is already documented, and, if combined with the understanding reached in the studies of the Bender group, might put us into the position of identifying the real neuronal circuits of the oculomotor system. By the term "real neuronal circuit" it is meant that individual neurons be identified according to their exact position, the course of their axons, the precise site and type (excitatory vs inhibitory) of their synapses, and with identification of the origins and types of synapses contacting the first neurons. In other words, it could and should be our aim to describe the immediate premotor and eventually the more remote oculomotor network in the same manner as has been recently done for parts of the spinal cord, cerebellum, etc. This goal is well within our reach today, largely due to the studies of M. B. Bender and associates.

Probably the most ingenious insight by M. B. Bender from a lifetime's engagement with clinical and experimental observations on oculomotor mechanisms is his concept of an "eye-centering system" (19). The quick phase of nystagmus has been the headache, a real "crux neurologorum", for the neuroscientists, both in regard to its origin and its mechanism. However, no one to my knowledge has attempted to find an answer to the somewhat teleologic question: "What is it for?" Until recently, the teleologic approach to the sciences was considered as almost philosophically "illegitimate". At times, however, it becomes very useful. Instead of the trivial traditional answer, namely, "to reset the deviated eye," Bender went one step further in his generalization. By assuming a basic, albeit anatomically widespread, mechanism for the centering of gaze, he was able to give a unified concept explaining a host of sometimes conflicting clinical and experimental data. Thus, at least for the time being, he offered a quite satisfactory interpretation for the quick phase of nystagmus. This general concept may be compared to another ingenious idea derived similarly from observations on oculomotor or optokinetic mechanisms. I am referring to the *Reafferenzprinzip* of v.Holst (20) or, as it is now called in English, the "corollary discharge." Of course, such concepts have the fate and disadvantage of creating an ever widening gap between the elegance of giving apparently satisfactory explanations to a set of otherwise inexplicable facts and the real mechanisms (in both cases neuronal) which have to be at their bases. However, I have more confidence in this respect for Bender's concept because of the possibilities mentioned in the preceding paragraph. As soon as the concrete neuronal network of oculomotor mechanisms can be defined, even if this is limited for the time being to its premotor portion, we will have a better basis to apply systems theory than at present, when only the phenomenologic approach has been so far applied. Irrespective of whether and how far further analysis will substantiate Bender's hypothesis, it is at worst an excellent working hypothesis for studies along the lines that I have tried to indicate.

As with other motor mechanisms, we are confronted today in the oculomotor system with two basic questions for which we seem farther from a solution than ever: (1) What is the ultimate source from which commands for shifts of gaze arise? (2) How is sensory information leading to shifts of gaze routed into the oculomotor channels? A recent Work Session organized by the Neurosciences Research Program (21) dealing with movement in general has raised again these questions in modern neurophysiologic terms and has tried to give answers—unfortunately with little success. The negative answer in the field of ocular movements was foreshadowed for over 20 years by the findings of M. B. Bender and associates. After their mentioned observations on the wide distribution of cortical and brainstem foci from which conjugated eye movements can be elicited, it is not surprising that the traditional concepts of initiation of voluntary eye movement from frontal oculomotor fields break down in the light of modern neurophysiologic studies (22). The importance of the cerebellum in oculomotor mechanisms (16, 23, 24) is being increasingly recognized and may soon quite fundamentally change our traditional views.

Regarding the second fundamental question, the work of M. B. Bender conducted in the late forties with H. L. Teuber (25) and resumed more recently (26) are of considerable relevance. Even if we accept the fact that the superior colliculi have no immediate role in executing ocular movements, these structures cannot be dismissed from the central structures that have to be involved in visuo-oculomotor localization and target-finding in space. On the contrary, it can be predicted that it is in the superior colliculi where our basic question No. 2 can be approached with the best prospects of success. The problem here is merely how (and which neuronal system of) the colliculi reach the immediate premotor system of the oculomotor neurons.

Finally, Bender and associates have very significantly contributed to our knowledge about the relation between changes in the state of consciousness and patterns of eye movements (27) as well as to that on eye movement during sleep (28, 29). These studies showed the existence of two distinct organizing mechanisms for eye movement: one for the wakeful state and the rapid eye movement period of sleep and another for the other phases of sleep.

The questions that have been discussed here are very highly and subjectively selected bits from the impressive body of material on oculomotor mechanisms in the work of Morris B. Bender, whose bibliography alone would occupy more space than this entire article. They are but a token of the great admiration that a humble neuroanatomist feels when surveying the results of a lifetime's engagement with the problems of ocular movement, exemplifying the best traditions of American clinical neurology by unifying the highest standards in the practical art of medicine and scientific sophistication, both in raising and pondering fundamental questions and in technical accomplishment of research.

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Pneumoencephalographic Demonstration of the Anatomic Basis of a Postencephalitic Korsakoff Syndrome

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Although the occurrence of severe memory defects following encephalitis has been described in the literature (1–12), in vivo demonstration of the anatomic basis for such defects has been rare. This report details the clinical and radiographic findings of a patient who developed a profound disturbance of recent memory following an acute encephalitis.

Case Report

A 56-year-old man was admitted to the Wadsworth VA Hospital November 18, 1971 because of an acute febrile illness associated with lethargy and confusion. Three weeks prior to admission, he had immunized some horses against equine encephalitis. Two weeks later, he developed a fever of 102°F and felt nauseated. At about the same time, the patient's wife noted a change in his behavior. He became very disorganized, irritable, and was intermittently confused. These symptoms persisted and several days later he was observed to be lethargic as well. On November 18, 1971, he was admitted to the Wadsworth VA Hospital.

There was no history of headaches, diplopia, photophobia, stiff neck, head trauma, seizures, alcoholism, or drug ingestion. Prior to the onset of the present illness, the patient's memory and general fund of knowledge was always good.

Examination on admission revealed a well-developed, well-nourished man who appeared somnolent but was in no acute distress. The blood pressure was 140/90 mm Hg; pulse, 88/minute; respirations, 20/minute; and temperature, 101°F. The general physical examination was normal. On neurologic examination the patient was awake but very confused and lethargic. Because of his clouded state of consciousness, only a limited examination of higher mental functions was possible at the time. He did not know the date, or the name of the hospital, and denied there was anything wrong with him.

There was no spontaneous verbal activity, and he answered most questions in one- or two-word sentences only. He could not remember the name of the president of the United States. He named most objects correctly but made occasional errors. There was no visual field defect to confrontation and the optic fundi were normal. The remainder of the cranial nerve examination was unremarkable. There were no focal motor or somatosensory deficits. Deep tendon reflexes were active and symmetrical, and there were no Babinski's. Meningeal signs were absent.

Hemoglobin was 15.1 gm per 100 ml and the white blood cell count 11,500/cu mm with a normal differential count. Electrolytes, blood glucose, urea nitrogen, and prothrombin time were within normal limits. A serum Venereal Disease Research Laboratory (VDRL) test was nonreactive and a heterophile agglutination test was negative. Chest and skull roentgenograms and a brain scan were all normal. Lumbar puncture yielded clear and colorless fluid under an initial pressure of 190 mm. The fluid contained 13 red cells/cu mm; 331 white

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cells/cu mm (328 lymphocytes and 3 neutrophils); 84 mg/100 ml glucose; and 138 mg/100 ml protein. Gram stain, India ink preparation and acid-fast stain of the spinal fluid were negative, and subsequent cultures of the fluid were sterile. Cytologic examination of the fluid for malignant cells was negative. Three repeat lumbar punctures performed during the first 2 weeks of the patient's hospitalization continued to reveal a predominantly lymphocytic pleocytosis with elevated protein and normal glucose. Complement-fixation virologic studies for herpes simplex, mumps, Eastern and Western equine encephalitis and California encephalitis, and hemagglutination-inhibition virologic studies for Venezuelan equine encephalitis, Saint Louis encephalitis, and yellow fever were performed on the patient's serum and cerebrospinal fluid during the second and fifth weeks of his hospitalization; although no elevated titers were demonstrated, it was felt clinically that a viral encephalitis was the most likely diagnosis.

The cerebrospinal fluid values slowly returned to normal and the patient's initial lethargy and confusion gradually cleared; however, he was left with a persistent, severe defect of recent memory in the presence of only mild impairment of other cognitive functions. Detailed mental testing 3 weeks after admission revealed that immediate auditory recall of digits and short sentences was quite good, as was immediate visual recall of simple designs and famous faces; however, when the examiner waited one minute and then called for a response, the patient could not remember a single item from any of the above tests. He was unable to recollect any of 5 unrelated objects after 1 minute and recall of short stories was impossible. Attempts to improve the patient's performance on the above memory tests by offering him monetary rewards proved unsuccessful. Although the patient did better on tests of remote memory, he still made frequent errors. He remembered the names and ages of his wife and four children, but could not recall the date of his marriage or important dates in American history. Disorientation for time and place persisted throughout his hospitalization but was not as severe as on admission. Although denial of illness was present initially, it had disappeared by the end of the third week. No confabulation was noted at any time.

Spontaneous speech was normal as was comprehension of spoken commands. Simple calculations were performed without difficulty and spelling was unimpaired. There was no right-left disorientation and he named body parts accurately. He correctly named objects on confrontation and in pictures and demonstrated the use of objects easily without evidence of dyspraxia. Reading and writing were normal, as was copying of simple designs. Proverb abstraction was difficult, with a tendency towards concreteness. Psychometric testing 5 weeks after admission confirmed the clinical impression that memory function was more severely affected than other cognitive functions. On the Wechsler Adult Intelligence Scale he obtained a verbal score of 97 and a performance score of 93. The full scale intelligence quotient was 95—within the average range. On the other hand, his performance on the Wechsler Memory Scale was severely impaired. Outstanding was the dissociation between the patient's almost complete inability to form new associations (shown by a score of zero on the associate learning test), and his normal performance on the digit span test which measures immediate memory. This pattern has been described as characteristic of patients with Korsakoff's psychosis (13).

Two months after admission, a pneumoencephalogram was performed (Fig. 1). The most striking finding was the marked enlargement of the temporal horns, particularly the left. This enlargement of the temporal horns was out of proportion to the mild to moderate dilatation of the anterior horns and bodies of the lateral ventricles. Most of the cerebral sulci appeared normal but a few were enlarged. The patient's course following the pneumoencephalogram has not changed. To date, the clinical picture remains essentially the same.

Discussion

Pneumoencephalography has been performed in only four previously reported cases of postencephalitic Korsakoff memory syndrome (4, 7, 8), and in only

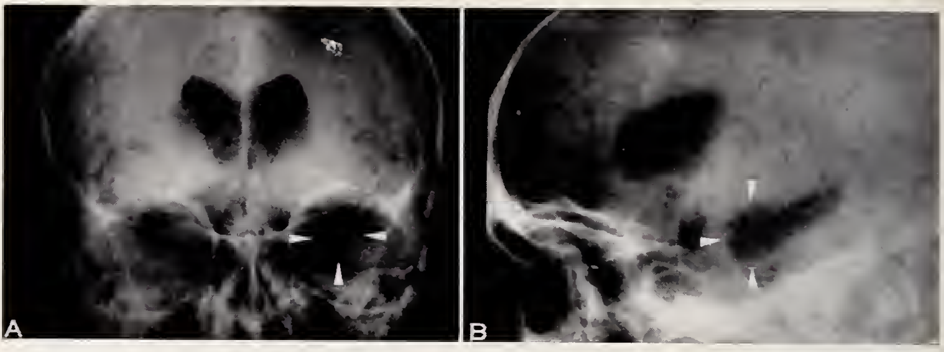

Fig. 1. Anteroposterior (A) and lateral (B) pneumoencephalogram films with patient in brow-up position. The considerable enlargement of the temporal horns, particularly the left one (arrowheads), is out of proportion to the mild to moderate enlargement of the anterior horns.

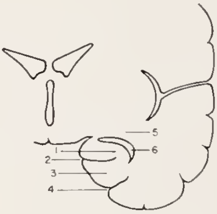

Fig. 2. Structures of the medial temporal lobe, including hippocampus (1), hippocampal fissure (2), parahippocampal gyrus (3), collateral fissure (4), region of the amygdala (5), and temporal horn (6).

one of these was a discrepancy noted between the relative dilatation of the temporal horns and the rest of the lateral ventricles (4). The pneumoencephalogram done on our patient clearly demonstrated a disproportionate enlargement of the temporal horns in the presence of relatively little enlargement of the rest of the lateral ventricles and showed only minimal sulcal enlargement. This appearance is consistent with a relatively selective atrophy of the structures of the medial temporal lobe (Fig. 2). It contrasts with the picture seen in diffuse cerebral atrophy where the pneumoencephalogram nearly always demonstrates more dilatation of the frontal horns and bodies of the lateral ventricles than the temporal horns (14) (Fig. 3). This has led to the use of relative enlargement of temporal and frontal horns as a means of differentiating between cerebral atrophy and hydrocephalus. Sjaastad et al found that relatively great enlargement of the temporal horns occurred in hydrocephalus, whereas relatively little occurred with cerebral atrophy (14).

In our case, the pneumoencephalographic findings are not consistent with hydrocephalus of either high- or normal-pressure type, according to the usual criteria (14, 15). The corpus callosal angle is not acute, the anterior horns are not ballooned, there is only a slight increase in the height of the anterior horns, and there is good filling of cerebral sulci. It would therefore appear that the disproportionate enlargement of the temporal horns in our case is related to a

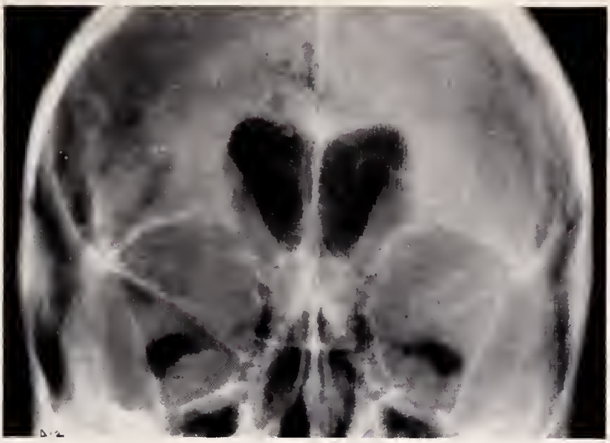

FIG. 3. Pneumoencephalogram of a patient with diffuse cerebral atrophy, showing the characteristic pattern of lesser degree of enlargement of the temporal horns than frontal horns.

selective atrophy of medial temporal lobe structures. This in vivo anatomic demonstration, together with the clinical data, points again to the importance of these structures for recent memory function in man.

Summary

A 56-year-old man developed a Korsakoff memory syndrome following an attack of acute encephalitis. Pneumoencephalography demonstrated a striking enlargement of the temporal horns which was disproportionate to the mild to moderate enlargement of the rest of the ventricular system. The pneumoencephalographic findings are consistent with a relatively selective atrophy of medial temporal lobe structures and, when correlated with the clinical data, would appear to provide radiographic documentation of the importance of these structures for recent memory function.

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The Neurology of Humor

EDWIN A. WEINSTEIN*

Among the qualities and accomplishments of Morris B. Bender commemorated in this volume should be his humor. Anyone who has attended Grand Rounds would agree that there is "never a dull moment"; and who can fail to recall, at the height of a loud, heated discussion, MBB's classic remark, "Who's yelling?" Other memorable features are the ludic dialogues with patients in Yiddish, terminated, unfortunately, on one occasion by the patient's comment, "I'm sorry, Dr. Bender, but I'm Italian." Worthy of note also, is the Benderian designation, "Jewish organic mental syndrome," i.e., the patient who answers a question with a question. MBB has a gift for nicknames and euphemisms based on the physical or personality features or provenience of the recipient, ranging from "der Mo" and the "Tank Commander" to "Big John" and "Big George." I can recall in the days when we were working with monkeys in the old Animal Room, a rosy complexioned young nursing student whom MBB would greet with, "How's my monk with the brilliant sexual skin?" Other references symbolize traits of pompousness and pedantry and woe betides any resident who cites the textbook to support his thesis. MBB is particularly fond of satirizing the various confabulations, rationalizations, and explanations of the proponents of heroic treatments that all too often speed patients down the road to "Andersonville."

My paper concerns the humor of patients, many of whom I studied jointly with Bender. In the denial syndromes (1), in the amnestic confabulatory states (2), and in jargon aphasia (3), there are patterns of symbolic organization that contain many of the features of humor. Usually, the patient with a severe aphasia or organic mental syndrome, does not have the vocabulary or facility with metaphorical speech to maintain the several levels of language on which humor depends. We are not apt to laugh or smile when an aphasic patient calls a pen a "pencil" or calls an elbow a "knee," but with the nonaphasic misnaming that occurs in the denial syndromes, a head bandage may be called a "turban," a urinal a "pissarino," and the side rails of a hospital bed a "starting gate." A patient referred to her paretic arm as "my little daughter," and various humorous designations are listed by Macdonald Critchley in another paper of this volume. In disorientation for place, we have had "Mount Cyanide" and I recall, in the days when a large staff retinue trailed the Chief on rounds about the ward, a patient who referred to the hospital as "Mount Schlepperman."

In confabulations, which are usually a metaphorical or allegorical representation of the patient's problem or disability, humor is frequent. One patient, when asked about his hemiplegia, attributed his problem to his wife and referred

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to his "better half." Recently, one of MBB's own patients with a left hemiparesis and hemi-inattention syndrome was reported by his wife as having shown no behavior disturbance, except that he was insisting on a rebate of half the taxes on his house because of depreciation! Another man with an old head injury, who had been committed to a mental hospital by his wife, would tell the story of having gone to see the film "Samson and Delilah" when asked about his injury. Colloquialisms and clichés may appear as humor in the instance of a man with a subarachnoid hemorrhage, who stated that "I was just minding my business and waiting for a trolley car when. . . ."

In reduplication, a patient may say that there are two or more Mount Sinai Hospitals, which differ in some feature germane to the patient's problem. A patient who would become disturbed at night stated that there were three Mount Sinai Hospitals, one at its proper location and the others "out on the (Long) Island." She complained that while the nurses in "this" hospital paid no attention to patients, those in the "other hospitals" were kind and attentive. Moreover, one of the "good" hospitals was owned by her nephew. Not only the hospital, but their personnel can be duplicated, as in the following interview with a Jewish lady from the Bronx, recovering from a subdural hematoma. Initially she had claimed that there were two Mount Sinai Hospitals, one at its actual location and the other in the Bronx.

Q.—What is the name of this place, Mrs. L?
A.—Mount Sinai Hospital.

Q.—Where is it?
A.—On 100th Street.

Q.—Are there any other Mount Sinai Hospitals in New York?
A.—Well, part of it is a hospital and part is a shul.

Q.—What do I do here?
A.—You're a doctor, you work here.

Q.—Do I work in the other part, too?
A.—Sure.

Q.—What do I do?
A.—Daven, of course, what then?

The behavior of some patients with denial has many of the features of comedy. Here the term, *ludic*, used by Jean Piaget (4) to describe the play, pretending and imitating behavior of young children is apt. Such patients caricature their disabilities and talk about them in melodramatic fashion. They frequently imitate the examiner's acts or parrot his words. They commonly respond to a query about their condition with a question about the doctor's health. Probably all neurologists have encountered the patient who, when asked how he feels, replies, "With my hands." A variant of this response occurred when I approached a well-developed blonde lady who had, unfortunately, acquired a lead encephalopathy from chewing paint. When I asked her how she felt, she answered in a euphoric fashion, "Feel me and find out for yourself."

In both the denial syndromes and classic comedy, there are the mistaken identities, the dual roles of characters, and the confabulated explanations in coping with difficult situations. Incidents which are initially trivial or traumatic undergo a transformation of meaning in the course of plot development. For example, a woman of 25 years was hospitalized because of a torticollis and a bizarre gait, associated with dystonia musculorum deformans. She complained of severe pain in the back of her neck, associated with the forced turning movements of her head. On admission to the hospital she was depressed and refused to leave her room because she was ashamed of her gait and other involuntary movements and was embarrassed, lest other patients watch her. Because of her depression and pain, she was given a course of electric shock treatment. After the third or fourth treatment she became disoriented, calling the hospital "Mount Sinus." Her movements were unaffected but she became euphoric and had no hesitation in conspicuously walking about with an even more bizarre gait, featuring a hip wiggle. She now claimed she was being watched because she was so "sexy," and that other patients on the floor who wore cervical collars, like her own, were men in disguise.

Other patients enact a tragic role. While the comic patient caricatures illness, they imitate death. Disorientation takes the form of the patient locating himself in a prison or graveyard, as in "Mount Sinai Cemetery." Some patients appear to "play dead," with a clinical picture of akinetic mutism as they rarely move, do not speak or talk in a barely audible fashion, refuse to eat or swallow, or do not seem to feel pain (pain asymbolia). Two of our patients actually claimed that they were dead. At times it may be difficult to separate the comic from the tragic, or the euphoric from the paranoid, as in the instance of one patient who did not think she was ill but charged that she had been beaten up by the residents.

Patients often give self-referential interpretations of idioms and proverbs in which a reference to their predicament is incorporated in the response. Thus a man with speech difficulties interpreted the expression "caught flat-footed" as "I wouldn't want to be caught with my foot in my mouth." Another patient with jargon aphasia responded to "birds of a feather flock together" with "people of similar dissitudes copulate together." Similarly, they give self-referential interpretations of jokes. We have used a Webster cartoon in which an ensign showing a girl over his ship, points out the spot "where our brave captain fell," whereupon the girl responds with "no wonder, I darn near slipped myself." While patients may recognize the double meaning, they say, "she might have hurt herself," and "there's nothing funny about falling."

Jargon aphasia combines features of aphasia and the denial syndromes. We have reported the case of a young soldier with jargon (5), who not only denied there was anything wrong with his speech, but claimed he could speak "French, English, Japanese, and Kentuckian." Here is a dialogue with another patient.

Q. — Is there anything wrong with your speech, Mr. O.?

A. — Well, I've been balancing my brains in the language division pending the outcome and I make these ideo statements for the benefit of the faculties that were checked in and out.

Q. — What bothers you now?

A. — Fission, the old charades but the rigor of the next week twentieth will be the stingy of the electris so you can see just how many you want.

Does the patient know he is being humorous? This question is similar to asking whether the disoriented patient really knows where he is or isn't or whether the patient with denial of illness really knows that he is ill. It is obvious that the woman who called the hospital "Mount Cyanide" had some knowledge of the correct name in order for her to have made such a play on words. For some disoriented patients, then, the knowledge of the correct name of the hospital is actually necessary for humor to appear. Also the disoriented patient may name the hospital correctly in one context and not another and persist in his misnaming despite cues, clues and corrections, much as a joker perpetuates a favorite joke. Also humor, like denial, is a product of a dyadic interaction and each appears in an interpersonal context. Moreover, the same behavior may be experienced as humor in one setting and not another, and by some observers and not others. One patient persistently called one of our wealthier residents, "Mr. Fishman from Fishman's Fish Market," despite his (the resident's) fervent denials. Here, the issue arises as to who was funnier, the patient or the resident.

Humor and the denial syndromes have other features in common. In each, language serves as a mode of adaptation to the stress of an unpleasant or traumatic reality. There are the sharp contrasts of linguistic content and pattern and a rapid shift from one to another. The transition is well shown in jargon aphasia. The patient with jargon speaks nonsense but he speaks it with normal rhythm and intonation. No patient with jargon aphasia talks jargon exclusively and we hear a sudden change from normal speech to gibberish. In humor and the symbolic patterns of confabulation, disorientation and reduplication, the same or similar content is used in multiple frames of reference. There is a juxtaposition of similarity and difference, as in the alliteration and antithesis expressed in the name, "Mount Cyanide." In a referential sense, "Mount Cyanide" is meaningful by reason of the approximation to "Mount Sinai." In an experiential context, it has meaning in terms of the patient's feelings about the doctors and nurses. A sense of meaning also arises from an identification with the values, traditions and argot of a particular social or ethnic group, as indicated by the response, "Mount Schlepperman." In its structural aspects, the quality of humor seems to derive from the relation of a symbol to multiple referents in multiple contexts. The experience of the speaker or actor is one of intense *meaning* or, perhaps, superordinate truth.

The behavior that has been described occurred under hospital conditions and routine methods of observation, only in patients with certain types of brain dysfunction. This does not mean that the brain lesion in itself "causes" humor. Nor are such explanations as "confusion," "loss of insight," and "release of repressions" helpful. We are dealing with highly patterned behavior, involving complex symbolic integrations and processes of interaction in the environment of which one is largely unaware. The humor of brain-injured patients is not intrinsically different from that of the normal, i.e., non-brain-injured person, in a situation of stress. It is likely that the transient neuro-endocrine changes occurring in stress and extreme emotion are analogous to the changes produced by dysfunction on a structural basis. The difference is one of degree; in the choice of the situation in which humor is used, in the degree of awareness of multiple frames of reference and levels of interaction in the environment.

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The Isolated, Intraarterially Perfused Frog Spinal Cord: An Improved Preparation for Physiologic and Pharmacologic Studies

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It is a great honor to be able to contribute to a collection whose purpose is to express appreciation for the contributions of Dr. Bender. We were students of his in the first (R.W.) and second (H.M.) generation. We hope that in some way we have learned to capture the elusive combination of spirits that characterizes him for us: a joy and vigor that is not incompatible with an inquisitiveness that fruitfully questions even the apparently obvious and a critical element which, although ready to attack, never discourages the young.

The spinal cord was the first area of the central nervous system to be studied with intracellular techniques (1). Despite the intensity of effort invested in electrophysiologic and pharmacologic studies of the spinal cord in the last decade (2, 3), a number of important questions have yet to be answered to complete our understanding of this important region of the central nervous system. Moreover, the spinal cord continues to serve as a useful model for understanding processes in higher centers (3, 4, 5). The difficulties in physiologic and pharmacologic studies of the central nervous system partially derive from the large number of elements involved and the complexity of intercellular organization; no small part of the problem, however, results from the relative inacessibility of important structures and the great sensitivity of these tissues to environmental changes. General patterns of organization, on microanatomic as well as gross levels, do, however, recur from animal to animal, and the proper identification of coherent inputs (6) allows for a high degree of experimental reproducibility.

It is of interest that, when Eccles et al (7) were not able to demonstrate an unequivocal role for potassium ions in spinal disynaptic inhibition, they suggested that the use of an amphibian preparation might provide an answer to this important problem. The ruggedness of amphibian tissues in comparison to those of mammals is almost proverbial. Thus it appeared possible to maintain amphibian spinal cord in a functionally active state during the changes of extracellular potassium ion concentration necessary for the desired demonstration.

In fact, intracellular recording from amphibian spinal cord began at approximately the same time as in mammals (8). Although the physiology of mammalian spinal cord has been more extensively investigated than that of the amphibian, investigations have revealed the presence of several important

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advantages in the use of the amphibian spinal cord. One outstanding example is the segregation of segmental monosynaptic excitation to motoneuron dendrites while monosynaptic suprasegmental excitation is localized on the soma* (9). Since the absence of predictable somatic excitation in mammalian motoneurons has been a major obstacle in the identification of spinal cord excitatory transmitters (3, 5), the anatomical arrangement in the frog suggests the use of the amphibian preparation to identify excitatory spinal transmitters.

Pharmacologic studies utilizing amphibian spinal cord have been characterized by a number of different approaches. Each of the preparations used appears to have advantages and disadvantages. The outstanding problems which have dictated the choice of preparation include: 1) inaccessibility of the structures to be studied, with its corollaries—very long times to steady-state and unsatisfactory oxygenation; 2) tissue damage and disturbances in structural organization; 3) movements which interfere with stable intracellular recording; and 4) distortions produced by effects on systems other than the spinal cord. A brief survey of the types of preparation will demonstrate the interrelationships among these various problems.

The use of the spinal cord in the intact frog has been attempted in several variations. The advantage of this approach is minimalization of tissue damage by using the animal's own respiratory and circulatory systems. Movements associated with respiration may be a severe limitation for intracellular work with such a preparation; and the remoteness of the tissue to pharmacologic access is another disadvantage. One attempt to bring the preparation closer in a pharmacologic sense has been to superfuse the spinal cord with a defined physiological solution for collection of materials from the cord and for delivery of pharmacologic agents (10–12). Carlson (13) attempted to use the perfused intact vascular system, substituting the heart action with a pump, but encountered the problems of general systemic effects of pharmacologic agents as well as great dilution and moderately slow equilibration times.

The use of the isolated spinal cord (9, 14) solves the problem of movement, but O_2 diffusion through the pia into the spinal depths is slow and steady-state reflex responses are appreciably reduced in these preparations. In addition, perfusion of pharmacologic agents from the bathing solution is a very slow process—simple inorganic ions taking 15 to more than 60 minutes to reach extracellular steady-state condition (14). The probability of maintaining intracellular recordings during more than one solution change is very small indeed.

In order to overcome the problem of diffusion, a delightfully simple preparation has been developed of the sagittally hemisected amphibian spinal cord (15). This preparation offers great mechanical stability, quick pharmacologic equilibrium, and absence of remote influences. The major disadvantage of this preparation is the probability of tissue damage, especially as the extensive motoneuronal dendritic tree in the amphibian approaches the mid-line and even crosses it (Figure 1 of Brookhart et al (16)). Despite the damage produced by hemisection, the preparation is remarkably stable, exhibits reflex activity, and has been the object of a large number of studies. (Among the outstanding exploitations of this preparation are the papers of Davidoff (17, 18) which go a long way toward demonstrating that GABA is the major transmitter involved in presynaptic inhibition. He has also, demonstrated that alcohol potentiates the action of GABA (19). Unaccountably, almost no intracellular recording has been reported using this preparation.

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* In contrast to what is usually taught, recent studies in comparative physiology indicate that ascent of the phylogenetic scale is not accompanied by a decrease in segmentalization. In fact, the opposite appears to be closer to the truth; development of functionally more sophisticated limbs is accomplished by a richer segmental reflex repertoire. On the other hand, control from above (usually by inhibitory pathways) is concomitantly increased.

It would appear desirable to have a preparation which has all the advantages of the hemisected cord but retains bilateral organization and avoids local tissue damage. Thus, we were struck by a report (20) of successful maintenance of an isolated bullfrog spinal cord by perfusion through the anterior spinal artery. After cannulation of the artery, physiological solutions are delivered by drip under hydrostatic pressure allowing flows of 4–22 ml/hr. Pharmacologic agents were introduced by injection into a cannula close to its entry into the anterior spinal artery.

We describe a successful modification of this technique which allows stable intracellular recording during a large number of changes of solutions. The solutions contain known concentrations of pharmacologic agents, and steady-state as well as kinetic dose-response relations can be obtained.

The Preparation

Specimens of the small frog found in Israel, Rana ridibunda, are cooled in ice until anesthetized and then decapitated. In order to remove the cord quickly with a minimum amount of damage, the following dissection procedure was developed. The ventral approach suggested by Brookhart et al (21) was utilized and the dissection carried out under oxygenated, streaming physiological solution. Figure 1a shows the appearance of the ventral aspect of the vertebral column after removal of the abdominal and thoracic contents. Using a dental drill with a fine saw the vertebral centra are removed and the cord and roots are exposed in situ (Fig. 1b). After the small roots are severed and the longer caudal roots (9, 10) are freed from surrounding structures, the cord and meninges are lifted from the laminae and placed in a separate bath (Fig. 1c). This portion of the dissection is accomplished in 20–30 minutes following decapitation.

In order to cannulate the anterior spinal artery, polyethylene tubing of 3 mm O.D. is drawn to a gradual taper over a very weak flame so that approximately 2 mm of the end is slightly smaller than the arterial diameter of 0.2 mm. A small drop of epoxy is placed on the tubing 2 mm from the end and serves as a guard both for tying the cannula and for handling with watchmaker's forceps to prevent mechanical obstruction of the fine-walled tubing.

After removing the dura, a small pinhole is placed in the anterior spinal artery several millimeters from its rostral end. The cannula is inserted into this hole and advanced to the drop of dried epoxy (Figs. 1d, e). A fine cotton thread is used to tie the cannula in place. Perfusion is then instituted and the preparation is mounted in a narrow groove in a perspex chamber with the desired roots drawn up into suction electrodes (22) for recording or stimu-

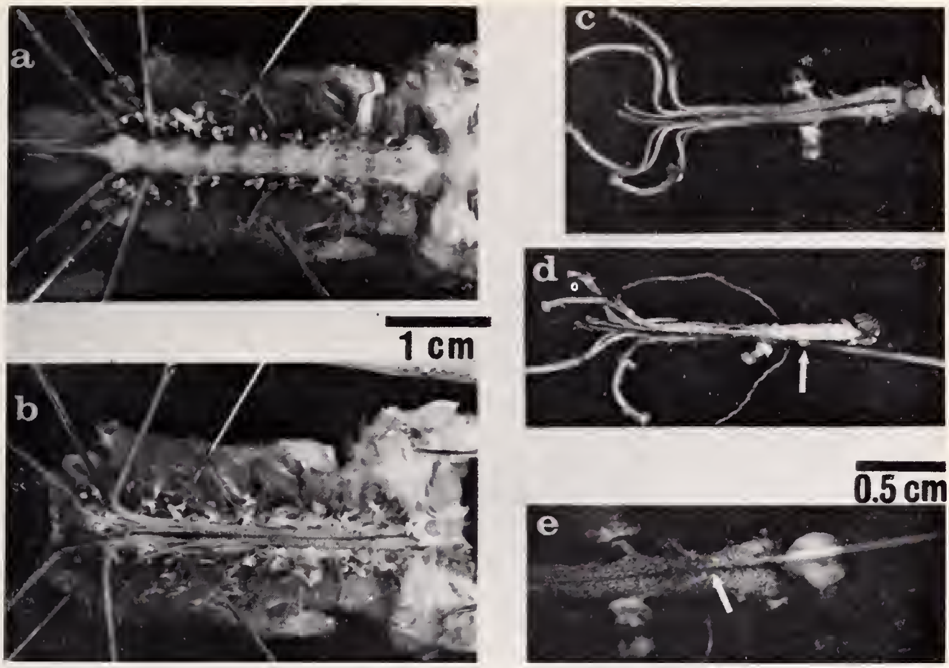

Fig. 1. Stages in dissection of the frog spinal cord and cannulation of the anterior spinal artery. a. Exposure of the spinal column and larger nerves (9, 10) from ventral aspect. b. After removal of centra, the anterior aspect of the spinal cord is visible. c. The cord, after isolation. d. The anterior spinal artery is cannulated. The rostral aspect of the cord is rotated and the epoxy drop (arrow) and cotton thread are visible. e. Enlarged view of the area of cannulation. The dark region of the anterior spinal artery (about 0.2 mm) is the region containing the cannula. The calibration of 1 cm refers to a–d; that of 0.5 cm refers to e.

lation. The solution in the bath flows at a rate of 3 to 5 ml/min and is identical in composition to that used by Grinnell (23).

The same solution is used for perfusion of the anterior spinal artery—the absence of arterial perfusion results in a slow deterioration of the preparation. The solutions are equilibrated with 95% O_2, 5% CO_2 for two hours while cooled in ice. The bubbling slowly reduces the pH of the solution to approximately 6; the pH is then raised to 7.4 by the addition of NaOH (producing an increase in Na^+ ions of about 2% over that of the original solution). The solutions are kept cooled by ice to prevent escape of the gases and to maintain the metabolic condition of the spinal cord at a low level. Perfusion is accomplished by hydrostatic pressure, and flow rates of less than 3 ml/hr are satisfactory to maintain the preparation in good condition for many hours.

Exchanges of solutions that are to be perfused through the anterior spinal artery are accomplished by a system of five rubber hydrostatic drips with air traps which are inserted into an expansion of the cannula. The rubber drips can be opened or closed by careful use of spring clamps to minimize mechanical shock, and exchange of perfusion solutions can be accomplished in under one second. The final mixing volume before entry of solutions into the anterior spinal artery is less than 0.006 ml.

Microelectrodes are introduced into the spinal cord after careful local disruption of the pia under binocular microscopy. The most satisfactory electrodes are those filled with 2 M potassium citrate, with resistances of 15–25 megohms.

Exploitation

When reflex discharges were recorded from the roots, active materials of known concentration could be introduced into the anterior spinal artery and dose-response relations were obtained. Thus, in a typical experiment, the VR-DR (ventral root-dorsal root potential), a manifestation of depolarization of dorsal root fiber terminals, was monitored. When glycine in concentrations of 0.75 and 1.5 mM was perfused, no effect was seen. On the other hand, 1.5 mM GABA produced a greater than 90% reduction in the VR-DR, reaching steady-state in under 60 seconds. After washing, recovery was complete in less than 60 seconds. As noted before, the preparation was useful for many hours without showing signs of deterioration. In general, steady-state for root recordings after solution changes was achieved in 20 to 60 seconds.

Intracellular recordings were accomplished without exceptional difficulty and stable recordings maintained for periods up to 90 minutes. Resting potentials of motoneurons up to 70 mV and antidromic spikes of 90 mV were obtained. In general, steady-state after a solution change was reached in 20–40 seconds. Thus it is possible in this preparation to carry out a large number of experiments on a single cell while recording intracellularly.

An example of a complicated pharmacologic experiment with a number of solution changes is given in Figure 2. Stable experimental conditions were maintained in this cell for more than 25 minutes and nine solution changes are illustrated. In A and B, representative antidromic spike heights and resting potentials from photographs taken every second are shown. At time zero, GABA 1.5 mM was flowed into the anterior spinal artery. After 16 seconds, failure of S-D (soma-dendritic) spike electrogenesis was noted (see Figs. 2C-b). Perfusion of the solution was maintained and a small hyperpolarization was noted but antidromic invasion into the soma spontaneously recovered, possibly indicating receptor desensitization. At 81 seconds, perfusion with a solution containing both glycine 1.5 mM and the same concentration of GABA was begun. A small depolarization was noted after ten seconds and, after another 9 seconds, block of antidromic invasion again occurred without recovery. When glycine alone was perfused, hyperpolarization was again seen. Return to GABA again produced a spontaneous recovery of antidromic invasion. Now washing with Ringers solution did not produce a substantial change. Repeated perfusions show the reproducibility of the results. The prolonged perfusion with glycine beginning at 1037 seconds shows a much slower desensitization-like process than that seen with GABA and may represent intracellular ionic re-equilibration. Representative oscilloscope traces showing successful and unsuccessful antidromic invasion are shown (Fig. 2C). On a dose basis, glycine inhibitory effects on motoneurons were invariably greater than those produced by GABA. These findings and similar experiments suggest that the two drugs act differently on the motoneuron membrane.

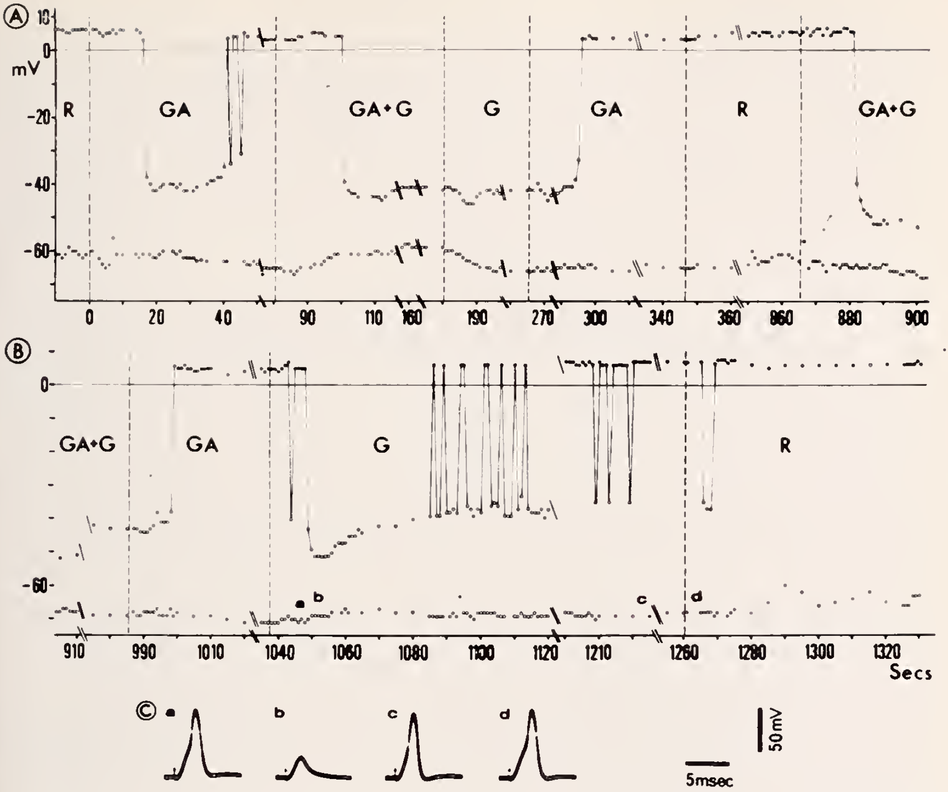

FIG. 2. The actions of glycine and GABA on the resting potential and antidromic spike of the same frog motoneuron. These potentials were sampled at 1/sec. A and B are a continuous plot during nine changes of perfusion solution. The perfusion solutions were Ringers (R), Ringers + 1.5 mM GABA (GA), Ringers + 1.5 mM glycine (G), and Ringers + 1.5 mM GABA + 1.5 mM glycine (GA + G). The vertical dashed lines give the times of solution change. Vertical solid lines show periods of oscillation between successful and unsuccessful somatic invasion of the antidromic spike. Periods with little or very slow change were eliminated and are denoted by parallel line pairs. The lowest row of dots represents the resting potential at each time while the upper dots give the height of the antidromic potential produced by stimulating the ventral root. C shows samples of successful (a, c, d) and unsuccessful (b) somatic invasion and correspond to the points with the same lettering in B. The calibrations refer to C. See text for further explanation.

Discussion

Despite the differences in organization and sensitivity to trauma and anoxia between mammalian and amphibian spinal cord preparations already alluded to, one is struck by a general consistency and equivalence of the fundamental mechanisms underlying spinal activity in both preparations. Motoneuronal spike electrogenesis in the amphibian spinal cord (8, 24, 25) is quite similar to that in mammalian motoneurons (7). Similarly, "spontaneous" unitary activity in amphibian motoneurons (14, 22, 26) finds a remarkable parallel in findings from mammalian motoneurons (27). The anionic sensitivity of the conductance increase accompanying disynaptic inhibition has also been investigated in amphibian motoneurons (28) and has been shown to be almost identical to that found in cat motoneurons (29). Finally, strong evidence that glycine is the major postsynaptic inhibitory transmitter in mammalian motoneurons (5, 30–33) has been confirmed in amphibians (34–36). This history of close correspondence between spinal mechanisms in the two phylogenetic classes suggests that it is fairly safe to analogize to one class spinal mechanisms (but not organization) from data found in the other.

The perfused anterior spinal artery preparation appears to be ideal for those studies where the influence of extracellular factors is important. In addition to the usual pharmacologic studies of spinal cord function, other major areas of fundamental interest appear to beg investigation in this preparation. First, the ionic mechanisms of motoneuron spike electrogenesis, including the question of dendritic spikes (3, 37), has not yet been adequately investigated. Second, the refined techniques for proving that a suspected transmitter has actions which are identical with those of the physiologically released agent (6, 32, 38) could be easily carried out in this preparation. Similarly, the ionic mechanisms of synaptic activity would also seem to be more easily investigated here. In addition, this preparation seems well suited to the determinations of the stoichiometry of transmitter-receptor interactions already accomplished in peripheral synapses (39–41). Another promising approach is the use of this preparation with the completely dissected peripheral nervous system of the frog as described by Carpenter et al (42). Finally, the preparation may be suitable for demonstration of release of transmitter after stimulation of presynaptic nerves (3, 5, 38).

It is obvious that our exploitation of the preparation has only been preliminary. Although the amplitude of the I-S (initial segment) spike after failure of the S-D spike is a rough measure of conductance change, more exact measurements of conductance are needed to quantify and explain the underlying processes (39). The advantages of obtaining a known extracellular concentration of material under study is important for quantitative studies and the extracellular concentration gradients in the spinal cord accompanying anterior spinal artery perfusion must be investigated.

When studying the effects of pharmacologic agents on single cells, the activity of presynaptic elements can be eliminated by raising the concentration of magnesium ions and/or by lowering calcium concentrations (14). In this way, by prohibiting transmitter release, the application is effectively confined to the cell under study.

Summary

1) A method is described for the pharmacologic analysis of spinal cord function in the isolated frog spinal cord.

2) The cord is maintained in stable condition for many hours by perfusion of an oxygenated Ringers solution through the anterior spinal artery.

3) Pharmacologic agents in fixed concentrations are delivered by the same route.

4) Stable extracellular (single-cell, field, or root) and intracellular recordings can be made during numerous solution changes.

5) Steady-state is usually obtained in under one minute.

6) The preparation will be exploited for solving problems in transmitter pharmacology and ionic mechanisms in the spinal cord.

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William Antopol Memorial Issue

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Editors' note. The following paper is not part of the tribute to Dr. Antopol, but it has been included in this issue in order not to delay further its publication. Parts II and III will be carried in the two succeeding issues of *The Mount Sinai Journal of Medicine.* However, the full list of references and the code for abbreviations will be included in each of the three parts for the convenience of our readership.

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WILLIAM ANTOPOL, M.D.

Preface

When anyone dies, those who knew him feel a touch of mortality. Beyond such self-concern we have reason to miss the wonderful man we knew as Bill. He was a great humanitarian available to anyone he could help or console, regardless of power or position. His professional career began at The Mount Sinai Hospital and ended as Professor of Pathology at Mount Sinai School of Medicine and Director of Laboratories and Research at the Beth Israel Medical Center. He could take on any topic without preparation, reflecting an inquiring, talented, and encyclopedic mind. He was a physician whose career was luminous in the compassion and brilliance of medical science.

The articles in this journal were written by his colleagues and illustrate the diversity of his interests. Because of space, the editors have been forced to omit the papers of many other gifted friends and colleagues. His original contributions on talc granuloma and sulfapyridine crystals in urine are now classic. Although primarily a morphologist, his interest extended to the physiologic and pharmacologic aspects of various pathologic conditions including endotoxic reactions, vasoactive polypeptides, and drug addiction. He gave the full use of his powers and excellence to the human condition.

One short sleep past, we wake eternally,
And death shall be no more

GEORGE BLINICK, M.D.*

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Lead Exposure: Focal Concentrations from Atmospheric Fallout

WILLIAM ANTOPOL, M.D., LEONARD NEWMAN, M.D., ROBERT DOERING, M.D., CHRYSSANTHOS CHRYSSANTHOU, M.D., AND EUGENE CRONKITE, M.D.

The late Dr. William Antopol was sincerely concerned about the welfare of the underprivileged child. He could not accept that lead in paint was the sole cause of lead poisoning and high blood lead levels in the children of the poor. As a pathologist he sought other causes. His preliminary observations showed astonishingly high lead levels in some New York City dust. To pursue the problem required large numbers of accurate lead determinations. He approached me to see if the analytical chemistry facilities of Brookhaven could help. There was willingness but the request came at the time of major budget cuts with concomitant reduction in staff. This was no deterrent to Dr. Antopol. He found the money from private sources in short order to support in part this joint effort.

This report is the first of what would have been a series of reports on lead contamination and lead poisoning. It is entirely fitting that this work be published in an issue dedicated to the memory and in the honor of Dr. William Antopol. (E. Cronkite)

While many current investigations are concerned with the lead content of paints, plasters, and atmospheric lead aerosols, relatively few reports deal with long-term accumulation of lead in the settled particulates of dust from atmospheric fallout. Considerable data are available on the lead content of the ambient atmosphere. Recently, the airborne lead was determined in the atmosphere of one of the busy automobile thoroughfares in New York City and daily averages of 7.5 γ per m^3 of air were found 6 meters above the street level which undoubtedly results in very high concentrations of lead in the fallout dust. Peak values occurred at 11:00 A.M. and 3:00 P.M. (1). In La Jolla, California, which is remote from industrial areas, lead makes up 0.09% of the fallout, corresponding to a daily lead deposition of 65 γ per m^2 of collecting surface. In Urban San Diego, however, the value reached 0.85% of the dust, corresponding to 950 γ per m^2 per day (2). It has also been found that the soil in urban parks is highly contaminated with lead and that the herbage from these areas contains significantly higher lead levels than herbage from rural pastures (3). In the annual snow strata in northern Greenland, the level of airborne lead has increased markedly since the Industrial Revolution (4).

The present investigation deals with atmospheric lead fallout which accumulates over prolonged periods on structures, such as window sills, which are directly exposed to thoroughfares and roadways. Thus localized reservoirs con-

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taining lead compounds can form at specific sites in concentrations and in absolute amounts which at times exceed even the highest atmospheric lead aerosol level by a factor of 100 or more. The particle size of most of the fallout is 6 μ or less. This corresponds with the reported particle size of lead in urban air (5). However, particles of 40 microns in size and occasionally larger aggregates have been observed.

Lead concentrations in such reservoirs were compared with the lead content in the particulate material which settled in exhaust ducts of the ventilating systems of two busy New York motor vehicular tunnels. This is designated as the "auto exhaust factor" * which serves as a baseline for comparison with the lead content of the atmospheric dust fallout; concentrations in these ducts range up to 10,000 ppm.

At times, differences between the lead content of the particulate material from the outer ledge of the window sill and tunnel dust are not very great. This is not entirely unexpected since outdoor paint (in which more than 10% lead is permissible) often flakes, crumbles and mingles with the atmospheric fallout.

High concentrates of inhaled lead from these reservoirs can gain direct access to the blood and tissues. This is particularly significant since lead blood levels in the general population already approximate those which may interfere with metabolic processes (4). In addition to the alveoli, where particle size plays a dominant role, inhaled lead aerosol can be absorbed from all portions of the respiratory tract including the nasal passages, because in these areas lead is solubilized by a mechanism which is as yet not entirely understood (6). Absorption is facilitated in the cases of soluble lead salts or lead-protein complexes. This is expected since some lead compounds which are insoluble in water still maintain high solubility in body fluids (7), a fact which may be critical in clinical plumbism. When high concentrations are absorbed into the circulation over short periods of time, a blood lead level which may produce severe clinical manifestations might be reached, especially with protein-bound and lipid-soluble compounds which enter the central nervous system.

Since the height above the thoroughfare, among other factors, is important, the lead content of outer sill dust was assayed at various floor levels of single buildings as well as buildings on both sides of an entire street. High concentrations of lead with wide variations, were found at different floors even in the same street, or for that matter on the various floors in the same building (Table I). Preliminary findings indicate the greatest lead fallout is present on the sills of the buildings at the center of the block and less on those nearer the street intersections (8). Obviously, lead fallout would be greater in narrow streets than on wide thoroughfares where the particulates are spread over a greater area.

For comparison with the auto exhaust factor, the lead content of window sill dust of some of the floors of the 12-story Beth Israel Hospital building on the northeast corner of 16th Street (westbound) and Perlman Place (northbound)

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* This would have been the subject of a separate publication except for Dr. Antopol's untimely death.

TABLE I

Lead Content of Dust Fallout from the Outer Ledge of Window Sills on Various Floor Levels of Beth Israel Hospital

The windows face southeast with a clear view from East 17th Street over the East River towards Queens.

ppm Pb	Window Location
6,400	14th floor corner
43,000	12th floor middle
I.S.	10th floor corner (insufficient sample)
3,600	10th floor middle
26,000	8th floor corner
50,000	8th floor middle
2,000	6th floor corner
5,900	6th floor middle
47,000	4th floor corner
43,000	4th floor middle
8,800	3rd floor corner
2,000	3rd floor middle
4,000	1st floor corner
4,100	1st floor middle

in Manhattan was taken for lead assay. Table I represents the lead levels in the dust which was collected from window sills facing towards the southeast. It is of interest that the distribution is somewhat erratic, indicating the unequal spread of the aerosol. Above the eighth floor, this building has an unobstructed view extending across the East River far into Queens, a distance of over 1 mile. Since the lead consumed by an automobile is emitted as fine particles which remain airborne for some time (9), the source of the aerosol probably originates for the most part from the local auto exhaust and to some extent also from distant sources.

An adult breathes approximately 15 m^3 air in 24 hours which contains from 3–5 γ lead per m^3 in light traffic areas to 6–9 γ per m^3 in heavy traffic zones, and from this the total daily absorption via the respiratory tract is 20–25 γ (4). In contrast, when exposed to the high lead levels in the reservoirs of dust above recorded (Table I), an individual might possibly inhale 1 mg or more of lead during several deep inspirations, depending on the percentage of the total particulate lead present in the dust. Even though the proportion absorbed is undoubtedly far less than the absolute amount inhaled, it can be expected that absorption via the respiratory tract will be greater than the 5–10 percent of ingested lead absorbed from the alimentary tract.

The observation that the brain lesions are more frequent in preschool children and occur most often from late spring into early autumn may have significance in that this corresponds to the period when windows are open and the preschool child's mouth and nose reach the level of the window sill or just above it (or the younger child in its mother's lap may also approximate the latter level), which creates a potential of inhaling concentrations of lead in a short period of time which are at least 100 times higher than the lead present in the total daily inhalation of ambient air. In view of this, it would be advisable to vacuum clean and then scrub the sills at frequent intervals. For that matter, the setbacks of buildings, the outer sill of fixed windows which do not open, as well as the area at the bottom of the walls, require similar treatment. This will minimize or eliminate not only the hazard of fallout dust, but also prevent re-entry of aggregated dust into the ambient air when the particulate reservoir is disturbed by winds of varying velocities.

Inhalation of such high concentrations of lead from these sources might be responsible for confusion and lack of expected meaningful relationship between the ingestion of lead-containing paint and blood lead levels, or even the combination of this with lead present in the ambient atmosphere before fallout occurs. In fact, there are patients in whom the source of high blood lead levels remains obscure; the sum of the lead in wall paint and ambient air being inadequate to account for the high blood findings.

Acknowledgments

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Amniotic Fluid Methadone in Women Maintained on Methadone*

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Introduction

By the early 1960's, the Bernstein Institute (then known as Manhattan General Hospital) was becoming the largest hospital in the United States for the voluntary treatment of heroin addiction by detoxification. In 1964, Dr. Ray E. Trussell, then Commissioner of Hospitals for New York City, suggested that the Beth Israel Medical Center undertake the administration of the Manhattan General Hospital. Mr. Charles H. Silver, President of the Beth Israel Medical Center, and Dr. Alfred Richman, the Chief of Manhattan General Hospital, amicably concluded this agreement. In 1965, methadone maintenance was advocated by Drs. Vincent Dole and Marie Nyswander (1) for the treatment of heroin addicts. Subsequently, the Bernstein Institute had both programs, i.e., detoxification and methadone maintenance. This gave Dr. Antopol, who had long been interested in the pharmacopathology of opiate drugs, the opportunity to set up not only methods for drug testing but also clinical and laboratory experiments in which he had long been interested (2). Originally, the program was limited to men because it was thought that women were too difficult to manage. But in 1968, women were finally admitted to the methadone treatment program, giving the Department of Obstetrics and Gynecology of the Beth Israel Medical Center a spectrum of observation in the diagnosis and treatment of heroin addiction. Despite folklore to the contrary, pregnancies have been observed in these patients and have been the subject of research which has been published and abstracted.

In the detoxification program, the addict is given an initial oral dose of methadone compatible with the amount of her habit, usually 20–40 mg. The methadone dose is gradually decreased until no medication is given. The detoxification treatment is designed to avoid severe symptoms of withdrawal from heroin and is very successful in this regard. After complete withdrawal, rehabilitation and psychiatric services are offered but invariably refused. Detoxification as a program, therefore, does not result in cure of the heroin addict and universal relapse and frequent repeat admissions are the result.

The Methadone Maintenance Treatment Program is aimed at the voluntary rehabilitation of the hard-core addict. The patients are given methadone in

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gradually increasing doses to induce a state of tolerance. The daily dosage is 5–10 mg initially and by gradual increments is raised to an average of 100–120 mg which provides maintenance by "narcotic blockade." At this point, heroin becomes neither necessary nor desirable and the addict returns to a normal, useful life. Initially, all patients were hospitalized at the Bernstein Institute and were very carefully screened to exclude alcoholism and schizophrenia, which are common factors in the addict world. Under these circumstances, of the eight babies born in this regimen, withdrawal symptoms were minimal (3). Because of the increasing amount of addiction, however, the program was expanded to a very large degree and now includes 15 hospitals and 35 clinics. It has become evident that close observation and supervision are not as successful as when the program began.

The impact of narcotic addiction on human reproduction has been the subject of considerable debate (4). Evaluation of the effects of opiates, including methadone, in the neonate has been based solely on clinical observation (5, 6). A direct biochemical measurement of the concentration of methadone in human fetal fluids has not been reported. The increasing number of pregnant women taking methadone in clinics or "on the street" makes such biochemical investigation desirable.

Using radioisotopically labeled opiates in pregnant rats, measurement has indicated that opiate drugs cross the placental barrier to the fetal tissue, including the fetal brain (7, 8). The use of radioactive methadone in human and fetal experimentation is undesirable. In humans, the difficulty involved in the direct biochemical assay of opiate drugs is due to low concentrations of drugs in the fetal fluids and the binding of morphine and methadone to human plasma albumin (9). Dr. Antopol, at the time of his death, was actively interested in developing techniques which would separate the bonds of these drugs to human plasma proteins (10).

He reported that the influence of proteins on the analysis of morphine by gas chromatography was studied by three types of experiments: dye-binding, hydrolysis, and dialysis. The results of these experiments indicated that serum protein binds with morphine sulphate and that such binding interfered with the analysis for morphine in the laboratory. The development of a specific and sensitive method for the quantitative determination of small amounts of methadone has made possible the measurement of methadone in fetal tissues during and after pregnancy.

Method

Amniotic fluid was obtained in 18 cases by amniocentesis or the vaginal collection of clear amniotic fluid at the time of rupture of membranes. Seventeen of these patients had been in the Methadone Maintenance Treatment Program for heroin dependence from one month to eight years at the Bernstein Clinics of the Beth Israel Medical Center. One patient was using methadone and heroin "on the street."

Amniotic fluid was obtained in 16 patients to determine the parameters of fetal maturity, i.e., lecithin-sphingomyelin ratio, creatinine concentration, and the surfactant foam test. In two patients, termination of pregnancy by saline amniocentesis at 14–18 weeks permitted the collection of amniotic fluid in the second trimester. In addition to the tests for fetal maturity, an aliquot of 10 ml of the amniotic fluid was frozen at 15°F for analysis of methadone.

The technique for this determination has been previously described (11). This method employs solvent extraction and gas liquid chromatography and is sensitive to as little as 0.015 μg of methadone and its metabolites in plasma samples from normal volunteers given a single intramuscular dose of 10 mg of methadone. Similar findings are reported in male methadone maintenance subjects.

Results

The basic clinical information concerning these cases is summarized in Table I. In the first eight cases, the measurement of methadone in the amniotic fluid was determined. In the ten succeeding cases, maternal blood was drawn concurrently so that the ratios of methadone in the amniotic fluid and the maternal plasma could be correlated. These findings are summarized in Table II. It can be seen that the concentration in amniotic fluid varied from 0.10 μg to 0.45 μg. In maternal plasma, the concentration varied from 0.14 to 0.48 μg. The ratio of methadone in the amniotic fluid to maternal plasma averaged 0.73, with a range of 0.39 to 1.56. At this time, it would appear that there is no constant relationship between the dose of methadone and the absolute levels of methadone in the maternal plasma, confirming prior data in male plasma (12).

Preliminary data of work in progress indicate a correlation between the levels of methadone in serum plasma and concentrations not only in amniotic fluid, but in cord blood, fetal urine, milk, and fetal tissues.

Discussion and Conclusions

These data presented document the transfer of methadone to the amniotic fluid of the human fetus. Since the level of methadone in the amniotic fluid in two patients undergoing termination of pregnancy from 14–18 weeks was similar to that found in amniotic fluid later in pregnancy, it is likely that transfer of methadone occurs during most, if not all, stages of pregnancy. The effects of prolonged intrauterine exposure to methadone are unknown. The newborns of methadone mothers have, so far, proved to be normal in their intellectual and physical growth (13). It has been repeatedly reported that one-third of the babies born to mothers maintained on methadone are premature by weight (14). This was true in our early series but in our more recent cases, prematurity by weight has not been as significant. With unreliable menstrual histories, the need for laboratory confirmation of fetal age in these patients is important. It is our impression that methadone maintenance, even with multiple drug abuse, stabilizes a woman's life, removes many stresses, and permits more adequate nutrition than the life of heroin abuse. These factors may very well be the reasons for more

TABLE I

Summary of Cases

No.	Age	FPAL	Onset of Drug Use	Duration of MMTP Prior to Conception	Gestation	Drug at Conception	Methadone Dosage Prior to Conception	Methadone Dosage at Time of Delivery	Multiple Drugs during Pregnancy	Infant Birth Weight
(1)	28	2012	1970	Street*	41 wks	Heroin	Street**	110 mg	Not Known	3856 grams
(2)	26	2002	1966	15 months	40 wks	Methadone	50 mg	50 mg	Not Known	3345 grams
(3)	40	0101	1950	12 months	40 wks	Methadone	120 mg	80 mg	Not Known	2736 grams
(4)	24	1112	1970	17 months	38 wks	Methadone	80 mg	80 mg	Not Known	2410 grams
(5)	22	1001	1969	Street*	40 wks	Methadone & Heroin	Street**	20 mg	Heroin & Cocaine	3544 grams
(6)	22	1001	1970	Street*	34 wks	Heroin & Barbiturates	Street**	40 mg	Heroin & Barbiturates	2197 grams
(7)	25	1001	1966	Street*	40 wks	Heroin	Street**	40 mg	Not Known	2948 grams
(8)	23	0100	1964	8 years	40 wks	Methadone	70 mg	70 mg	Heroin	2948 grams
(9)	23	0030	1960	6 months	40 wks	Methadone	90 mg	90 mg	Barbiturates	a) 1786 grams b) 1644 grams
(10)	20	1001	1969	1 month	14 wks	Methadone	80 mg	80 mg	Not Known	394 grams
(11)	26	2002	1966	3 months	40 wks	Methadone	90 mg	90 mg	Not Known	3187 grams
(12)	26	0010	1972	Street*	18 wks	Methadone & Heroin	30 mg	30 mg	Heroin	851 grams
(13)	22	0120	1966	5 months	40 wks	Methadone	100 mg	100 mg	Not Known	3735 grams
(14)	23	1001	1966	20 months	40 wks	Methadone	100 mg	100 mg	Not Known	3686 grams
(15)	21	1101	1967	Street*	40 wks	Heroin	60 mg	50 mg	Heroin	2722 grams
(16)	24	3013	1970	5 months	40 wks	Methadone	80 mg	80 mg	Not Known	3218 grams
(17)	19	2002	1969	9 months	37 wks	Methadone	80 mg	80 mg	Not Known	3062 grams
(18)	21	1011	1969	5 months	31 wks	Methadone	80 mg	90 mg	Not Known	1276 grams

* Duration unknown

** Dosage unknown

TABLE II

The Concentration of Methadone in the Amniotic Fluid

Case No.	Methadone Dose (mg/day)	Amniotic Fluid (mcg/ml)	Plasma (mcg/ml)	Ratio AF/PL
(1)	100	0.42		
(2)	50	0.18		
(3)	80	0.17		
(4)	80	0.45		
(5)	100	0.12		
(6)	80	0.10		
(7)	40	0.10		
(8)	70	0.15		
(9)	90	0.22	0.38	0.58
(10)*	80	0.28	0.48	0.59
(11)	90	0.25	0.36	0.70
(12)**	30	0.07	0.18	0.39
(13)	100	0.26	0.39	0.67
(14)	100	0.10	0.22	0.45
(15)	50	0.20	0.25	0.80
(16)	80	0.14	0.14	1.00
(17)	80	0.39	0.25	1.56
(18)	90	0.16	0.31	0.52

* 14 gestational weeks at time of abortion.

** 18 gestational weeks at time of abortion.

normal birth weights but the mechanism is still unclear. Whether later growth and development will be impeded or predisposition to addiction engendered cannot be answered at this time. The concentration of methadone in cord blood, fetal urine, and breast milk is under continuing investigation. The direct determination of methadone in fetal biological fluids and eventually in tissues may clarify the role of methadone in the withdrawal syndrome of the neonate. The role of multiple drug abuse in this withdrawal syndrome may be more important than previously appreciated.

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The Possible Implication of a Humoral Smooth Muscle-Acting Factor (SMAF) in Shock*

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Disparity between the circulating volume of blood and the volume capacity of the vascular system is the major factor underlying the reduction of blood flow and the resulting decreased perfusion and anoxia which sustain and further increase the circulatory deficiency in shock. Hypovolemia is a frequent but not a necessary condition for the development of the disparity between volume and capacity. Alterations in the physiologic state of hemodynamics other than or in addition to reduction of blood volume are significant pathogenetic factors. Marked increase in the vascular bed produced by dilation of the resistance and/or of the capacitance vessels could result in severe disruption of peripheral vascular homeostasis with subsequent circulatory collapse. Changes in vascular tone that could lead to the development of shock are primarily induced by humoral agents which are the main mediators governing the state of microcirculation.

A broad spectrum of humoral vasoactive factors has been implicated in the circulatory changes both of the early compensatory as well as the subsequent decompensatory phase of shock. Catecholamines, vasoexcitor material (VEM), vasodepressor material (VDM) (10, 19), myocardial depressant factor (MDF) (14), plasma kinins (3, 21) bacterial factors, prostaglandins (15) and other humoral agents (1, 2, 13) have been proposed as possible mediators of circulatory changes in shock. In view of the implication of vasoactive substances in the mechanism of shock it is apparent that humoral agents capable of modifying the responsiveness of vascular smooth muscle to vasoactive substances may play important roles in the pathogenesis of the disorder.

The present communication is intended as a preliminary report regarding the possible implication of such an agent in the pathogenesis of hemorrhagic and endotoxin shock. This agent, probably a polypeptide, is referred to as SMAF, the acronym derived from Smooth Muscle-Acting Factor. Originally found in mouse lung (6), SMAF, has been subsequently isolated from extracts of various organs (lung, kidney, placenta and blood) in several animal species (mouse, rabbit, rat, dog, human). The method for extraction and purification as well as the physical, chemical, biological and pharmacological properties of SMAF have

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been previously reported (8, 9). The properties of SMAF distinguish it from similar known substances including bradykinin, substance P, eledoisin, prostaglandin, factor BPF (11), Kutapressin (20), peptide B (12), and the polypeptides described by Said (16, 17).

The most striking action of SMAF, which is relevant to the subject of this report, is its ability to increase responsiveness of smooth muscle-organs (including blood vessels) to vasoactive substances (8, 9). SMAF also increases vascular permeability and elicits slow, weak contractions of smooth muscle (8, 9).

The possible role of SMAF in pathologic conditions has already been suggested in studies on the pathogenesis of decompression sickness (4–7, 9).

This report deals with the observed increase of the level of SMAF in the blood of animals subjected to endotoxin and hemorrhagic shock.

Methods

Endotoxin Shock: Thirteen female albino rabbits weighing 2–3 kg were anesthetized with intravenous administration of sodium pentobarbital (30 mg/kg body weight as an initial dose, with additional intravenous doses if and when needed to maintain anesthesia). Mean carotid blood pressure was continuously monitored by means of a Statham pressure transducer coupled to a Grass polygraph. Heart rates were calculated from ECG or blood pressure records. Endotoxin shock was produced by an intravenous injection of 1 mg/kg E. coli or Proteus vulgaris endotoxin. Arterial blood samples (10 ml) were obtained from all animals before (control sample) and after endotoxin administration. The latter samples were collected when the mean carotid blood pressure fell below 40 mm Hg, which usually occurred 1–6 hours after endotoxin administration. In some animals additional blood samples were collected at earlier stages of the hypotensive response before blood pressure reached the 40 mm Hg level.

Hemorrhagic Shock: Three female albino rabbits were anesthetized and the mean carotid blood pressure and heart rate monitored as described above. A cannula, connected to a plastic constant-pressure calibrated reservoir, was inserted in the carotid artery. The reservoir, tubing, etc., were all siliconized. Heparin (1500 U/kg) was intravenously administered prior to bleeding. The animal was bled into the reservoir in which the pressure was set to remain constant at 40–45 mm Hg by bubbling 95% O_2 + 5% CO_2 through the blood into the reservoir. After a variable interval following initiation of bleeding, blood flow was spontaneously reversed from the reservoir into the animal. When 40% of the shed blood was spontaneously reinfused, hemorrhagic hypotension was terminated by gradually raising the pressure in the reservoir to permit the remaining shed blood to be infused back into the animal. The purpose of gradual reinfusion was to avoid excessive rise in the central venous pressure. Preliminary experiments have shown that with the method described above, shock progresses to a terminal stage when the animal's mean arterial blood pressure declines below 60 mm Hg. Arterial blood samples (10 ml) were obtained before or at the time of initiation of bleeding (control sample) and during the hypotensive period which follows the reinfusion of blood.

SMAF Level Determination: The blood samples as soon as they were drawn were placed in 1N hydrochloric acid (0.3 ml of blood/ml acid) in a boiling water bath for ten minutes. SMAF was extracted and isolated by the previously reported methods for extraction of SMAF from tissues (9). The level of SMAF in the various samples was determined by measuring the optical density at 270 nm of the material extracted from 1 ml of blood and dissolved in 1 ml of water. Since the purity of the extracted material and consequently the validity of the optical density as an index of SMAF concentration can be questioned, the activity of SMAF in various samples was also determined by bioassays on isolated organs. In view of the observed correlation between SMAF activity and optical density, the latter was used as an expresssion of SMAF levels in the various blood samples.

Results

Endotoxin Shock: The initial immediate hypotensive response to the intravenous injection of endotoxin reported in dogs and other animals, which is followed by a transient recovery, was not observed in the rabbits used in these experiments. Following a varying interval after endotoxin administration the animals exhibited a rise in the heart rate and a gradual and progressive fall in the mean arterial blood pressure, which eventually led to the death of the animals. Table I shows SMAF levels (expressed in terms of optical density of extracted

TABLE I

Increase of SMAF in Endotoxin Shock

Rabbit #	Before Endotoxin		After Endotoxin		% Change in SMAF OD/ml blood
	B.P. (mm Hg)	SMAF OD/ml blood	B.P. (mm Hg)	SMAF OD/ml blood	
17140	96–104	0.050	20–30	0.245	+390
17141	92–96	0.020	30	0.140	+600
17142	100–112	0.084	0–40	0.140	+67
17143	108–112	0.054	20–30	0.110	+104
17095	120	0.026	30	0.048	+85
17096	120	0.015	50*	0.052*	+246
17111	100	0.070	†	†	†
17118	100–112	0.093	†	†	†
17119	92–108	0.081	30	0.069	−14
17120	92–104	0.069	28–30	0.131	+89
17121	100–108	0.081	44‡	0.106‡	+30
17128	82–108	0.090	20–30	0.076	−16
17129	110–130	0.120	30–40	0.143	+19

* The animal died before blood pressure reached 40 mm Hg and therefore there was no sample drawn at that stage. The sample drawn at an earlier stage, however, revealed a marked increase in SMAF level.

† The animal died before blood pressure reached 40 mm Hg. No samples were drawn at earlier stages.

‡ The animal revealed an increase in SMAF at an earlier stage when blood pressure had dropped to 44 mm Hg.

SMAF/ml blood) of the control blood sample (pre-endotoxin) and the sample drawn when blood pressure declined below 40 mm Hg. It can be seen that in 7 of 13 animals SMAF concentrations were appreciably increased when blood pressure fell below 40 mm Hg level. In two animals the increase in SMAF was manifested at an earlier stage and in another two animals the level of SMAF slightly decreased. The remaining two animals died before blood pressure reached 40 mm Hg and no samples were obtained at earlier stages. Excluding the two latter animals, the results indicate that SMAF levels increased in endotoxin shock in 9 out of 11 animals. Statistically evaluated, these results are significant at the P = 0.01 level of confidence (t test for paired observations). In those animals from which several blood samples were drawn during the post-endotoxin hypotensive period the level of SMAF showed a progressive increase.

Hemorrhagic Shock: Figure 1 is a representative chart showing the changes in the mean arterial blood pressure in relation to the hemorrhage and reinfusion. The figure also shows the level of SMAF (expressed in optical density of the extracted material/ml blood) in the blood samples drawn at the time of initiation of hemorrhage (sample #1, control) and during the initial (sample #2)

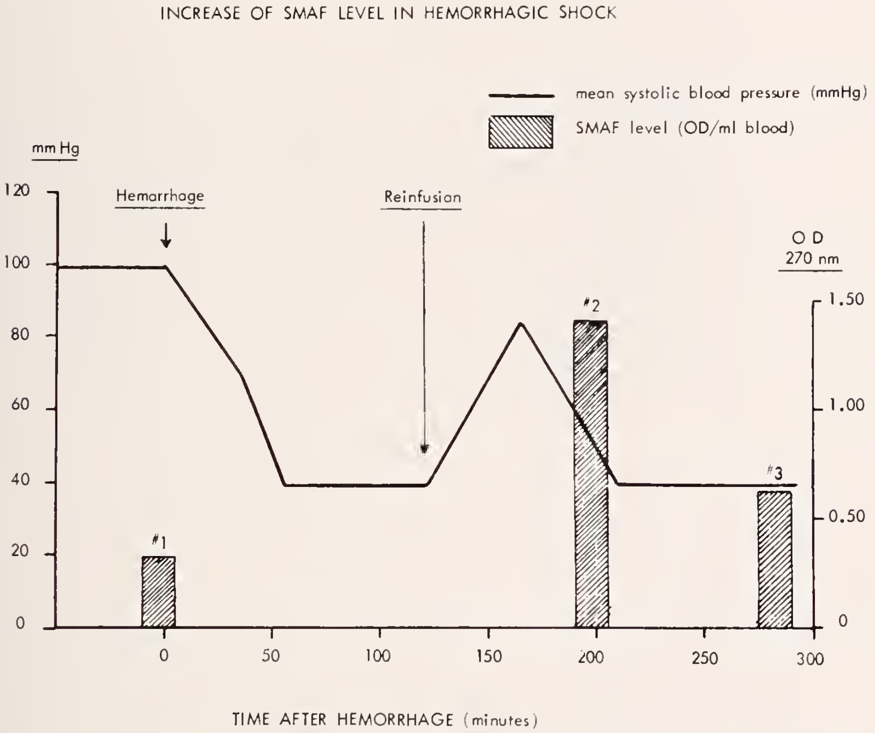
INCREASE OF SMAF LEVEL IN HEMORRHAGIC SHOCK

FIG. 1. Continuous mean arterial blood pressure recording with superimposed levels of SMAF in arterial blood samples drawn just prior to bleeding (#1), and in the early (#2) and late (#3) post-reinfusion hypotensive period. At the first arrow (Hemorrhage) the reservoir was opened and bleeding initiated. At the second arrow (Reinfusion) the shed blood was reinfused from the reservoir back into the animal. Note the increase in SMAF during the post-reinfusion hypotensive period.

and late stage (sample #3) of post-reinfusion hypotensive period. It can be seen that the level of SMAF in sample #2, drawn when blood pressure had declined to 50 mm Hg, shows a fourfold increase as compared to the control sample #1. In sample #3, drawn later in the terminal stage of shock and shortly before the animal succumbed, the concentration of SMAF, although it shows a relative decrease, is still above control level. In the remaining animals the results were similar, indicating an increase of SMAF level in the early post-reinfusion period with a relative decrease in the later terminal stage.

Discussion

The scope of the present investigation was confined to exploration of the possible release or activation of SMAF in experimental shock. The results of these preliminary experiments indicate that blood SMAF levels increase in the course of endotoxin and hemorrhagic shock. In the former type of shock the increase in SMAF appears to be progressive and in inverse relationship to the level of blood pressure. Regarding the hemorrhagic shock the available data suggest that SMAF levels increase in the early phase of the post-reinfusion hypotensive period with a relative decrease in the terminal stage.

Although SMAF has not yet been chemically identified, it exhibits chemical, physical and pharmacological properties (8, 9) which distinguish it from all known similar substances which act on smooth muscle. SMAF, probably a low molecular weight polypeptide, is capable of increasing responsiveness of smooth muscle-organs to stimulants. This property of SMAF may be of great significance regarding its possible role in physiologic functions and pathologic conditions. It can be postulated that the function of smooth muscle organs is regulated not only by neurogenic stimuli and myotropic substances which directly cause contraction or relaxation of smooth muscle cells but also by factors, like SMAF, which modify the capacity of these cells to respond to stimuli. SMAF has already been implicated in the pathogenesis of decompression sickness (4–7, 9). On the basis of the present preliminary investigation, the question of possible involvement of SMAF in the mechanism of shock can only be a matter for speculation. The possibility that SMAF, by increasing the reactivity of vascular muscle, may augment the effect of vasomotor stimuli in shock and thus contribute to the deterioration of the peripheral circulation is an attractive and provocative hypothesis. It is of interest in this respect that although depletion of kininogen was observed in several types of shock (18) and release of kinin during anaphylaxis was found in dogs (3), little correlation was seen between the severity of the shock and the amount of kinin formed (3). It is conceivable on the basis of the hypothesis discussed earlier that small increases in bradykinin levels and even "normal" concentrations of this vasodilator agent may induce significant vascular changes if the smooth muscle of the vessels has been sensitized by factors like SMAF with a resulting increase in responsiveness to bradykinin. In addition to the potentiation of the action of vasoactive agents, SMAF, which also increases capillary permeability (9), could cause extravasation of fluid and thus further increase the disparity between volume and capacity of the vascular system.

The stimulus and the mechanism whereby SMAF is released or activated in shock is at present unknown. Several possibilities merit consideration. It is conceivable that liberation or activation of SMAF is the end point or an intermediate link in chain reactions triggered by physiological and biochemical alterations in shock, such as decreased tissue perfusion and anoxia with disruption of lysosomes and release of proteolytic enzymes. Liberation or activation of intra- and/or extracellular enzymes with formation of chemical mediators from precursor substrates has already been reported in shock (10). It is also possible that SMAF may be released from tissues following ischemic injury, or activated by mechanisms similar to those which trigger the coagulation or kinin systems.

Summary

The level of a humoral Smooth Muscle-Acting Factor (SMAF) increases in the blood of animals subjected to hemorrhagic and endotoxin shock. The possible involvement of SMAF in the pathogenesis of shock and proposed mechanisms regarding its release or activation are discussed.

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Vocational Rehabilitation of Patients on the Beth Israel Methadone Maintenance Program

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Four years ago a vocational rehabilitation service was initiated at Beth Israel Medical Center. The need for such a service seemed obvious since ex-addicts usually are handicapped in job-seeking by their criminal records, lack of skills, and minority status. Yet at the onset the work of the vocational unit was curiously frustrating. The service was underutilized, few patients even being aware of its existence. Serious motivation for work seemed to be absent in many patients. They were often unreliable in keeping appointments, timid in going to job interviews, reluctant to accept the routine of menial jobs (which, for some, were all that were available), and apparently lacking in ambition. Nevertheless, most of the patients in the methadone treatment program professed a desire to work, and after a year of treatment the majority had actually obtained employment of some kind without the help of any vocational placement service.

Quite clearly, the role of a vocational service required reexamination. The need for the service was not in doubt since many of the unemployed patients were intelligent, ablebodied and no longer disabled by drugs, while a substantial fraction of the working patients were trapped in jobs below their potential. The social disabilities of the addict population under treatment went beyond the pharmacology of narcotics. What began as a routine service to ex-addicts thus has led to a deeper analysis of the disabilities in this population group and to a search for new techniques of social service.

Historically, vocational rehabilitation evolved to meet the special needs of the physically handicapped. From our present perspective this problem can be seen as relatively simple and well defined. A physical disability excludes some occupations but is compatible with others. With good medical treatment and imaginative vocational counseling, the patient with physical disability is made employable, and in many cases becomes an outstanding employee. An asset in this group, in addition to strong motivation, usually is a middle-class attitude toward work—a sense of responsibility in keeping appointments and fulfilling commitments, and a habit of upward mobility. As Hollinghead and Redlich (1) pointed out, these qualities are often lacking in the disadvantaged populations that they designated as socioeconomic class V, and it is in this group that social and psychiatric services are notoriously ineffective. A hard-working, upper middle-class person (such as a physician or a vocational counselor) will naturally be irritated by failure of a patient to keep appointments or to follow up on opportunities, but as pro-

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fessionals we must see the causes of this behavior as cultural disabilities which are not limited to addicts. The first step in improvement of social services to underprivileged persons is to overcome our own deficiencies in perception of the problem.

The ex-addicts, most of whom were born into disadvantaged social groups, are a population that would have had a high unemployment rate even without becoming addicted. They thus represent a combination of two disabilities—one cultural and the other a consequence of drug use. As addicts with drug habits starting in adolescence, they lost the critical years of social maturation. Education, job training, evolution of productive social attitudes were denied to them by the exigencies of the drug world and prison. The summation of cultural and developmental deficiencies in these patients might appear to make the vocational problem hopeless—yet the job counselor must hold faith in the potential of his patients. When discouraged he can look at the many thousands of ex-addicts who have overcome these handicaps and are now leading productive lives with steady employment. The problem is not at all hopeless, but it does need to be clearly defined.

An analysis of the first three years' experience of the vocational rehabilitation unit at Beth Israel Medical Center (2) emphasized the difficulty in applying traditional methods of vocational service to the rehabilitation of ex-addicts. Of 1,735 patients in the Bernstein unit of the methadone program, 350 were referred by their clinics for vocational counseling and 315 were seen at least once by a vocational counselor. A follow-up study showed that 122 of these patients subsequently improved their occupational status (either becoming employed if they were unemployed at the time of referral, or upgrading their job if employed when first seen). On the other hand, 193 of the patients seen by the vocational office subsequently showed no change or a decrease in the level of occupational achievement, failed to get any job or were discharged from the treatment program. Among the 122 patients who showed improvement in employment status after vocational counseling, some quite obviously were helped by the service, whereas in other cases the effect of vocational counseling was less clearly defined but presumably beneficial. Thus, taking improvement in employment status as a measure of the impact of the vocational rehabilitation unit, the facility was able to help only 7% (122/1735) of the patients during the first three years of its operation. This, of course, was only part of the service rendered by this office to the methadone treatment program since it also contributed to the education of the general counseling staff in vocational placement techniques and worked with employer representatives to widen job opportunities for all patients on the program.

A recent study of the characteristics of patients referred to the vocational rehabilitation office revealed no major bias in the age and ethnic selections (3). Comparison of 61 patients referred from two clinics with 41 other patients randomly drawn from the clinic records showed a slight excess of older patients in the referred group which, although statistically significant ($P < 0.05$), did not appear to be of practical importance in these small samples (e.g., 52 patients over 30 years of age referred; 47 expected in random selection). What clearly was important, however, were the varying judgments made by different program counselors in deciding which patients were suitable candidates for vocational counseling. The patients referred usually represented the extremes of clinic experience—either exceptionally well-motivated patients looking for vocational training or help in relocation to advance in job status, or the most unstable patients who were referred in the desperate hope that they might be salvaged by jobs. Thus, access to vocational services for the majority of patients was impeded by administrative arrangements that put the vocational services in a central office to which only selected patients were referred. Clearly, the vocational service must reach more directly into the clinics if the expertise of this resource is to have maximum effect.

In order to plan for the most efficient use of vocational services, let us analyze the process of job placement and then try to see what parts of this process might be best handled by the various staff members working together as a team:

(1) *Job readiness* is the basic condition of employability. It means that the specific disability of drug abuse has been removed by treatment and that the patient is prepared to seek employment. It should not be assumed that all this can be achieved simply by medical treatment and counseling. Persons who have experienced only failure and rejection in their lives may not have the perspective or the self-esteem needed to persist in job-seeking after unsuccessful interviews or in jobs after placement in menial tasks with pay at the welfare level. They need the continued support of a staff that understands their feelings, that is willing to leave the clinic when necessary and go with them to difficult interviews—that includes successful ex-addicts whose own lives demonstrate what can be achieved. The majority of patients do not need sheltered workshops, as some agencies have suggested, but they do need the backing of a concerned staff. Job readiness in a patient, therefore, means that he is now ready to function as a normal person in a position of responsibility. His drug abuse has been terminated; he is motivated and realistic in his plans; he feels strong because he is backed by the resources of a concerned staff that understands his problems. If he fails in his job, he will not be rejected for this reason, but helped.

(2) *Marketable skills* are often lacking in patients with a long history of addiction starting in adolescence. Physicians and counselors inexperienced in vocational work tend to see no alternatives for such patients other than welfare support or a menial job. But a recent survey (4) of work experience and interests of unemployed patients, conducted by the staff of the vocational rehabilitation service, disclosed a surprising variety of interests and skills which, if not fully operative at the present time, could rapidly be made usable by vocational training and well-chosen job experience. Patients, who are now unemployed, were found to have capabilities in clerical work, sales, factory and repair services, construction, personal services, transportation, professional and artistic work. Here, then, is the area in which the expert guidance of a vocational placement service is critically needed.

(3) *Job opportunities* may, of course, be limiting even when patients are ready

and able to work. Highly skilled workers with good work records are now unemployed in depressed urban areas. The patients, moreover, have to enter the job market disadvantaged by records of addiction (if known) and minority-group status. Every member of the staff must fight the pervasive discrimination against ex-addicts, and open the way for job-ready patients to prove their own value. The dilemma at the present time is that direct negotiations with industries to place patients may permanently stigmatize the employee as an ex-addict and thus in the long run make his position untenable. This need not happen, since the medical and personnel offices of most corporations are sensitive to the problem of confidentiality, and pilot studies now in progress are providing entry of qualified patients into responsible jobs. For the immediate future, however, the main channel of employment of ex-addicts undoubtedly will continue to be job finding without explicit backing or identification as ex-addicts. Many thousands of patients on methadone maintenance programs obtained their first job simply by ignoring the past, and by now have such good job records that they probably could continue employment even if, by chance, their past were discovered. This is not ideal, but it is the reality of today and emphasizes the obligation of all staff members to protect the confidentiality of the patient's clinical record except when, with his consent, the program sponsors him for placement in a specific job.

The expertise of vocational counselors is clearly needed in the development of job skills and opportunities. To identify the existing or potential skills of patients and to discover the myriad of opportunities for employment requires special competence in the vocational field. On the other hand, these services are ineffective if the patient has not been brought to the state of job-readiness, as above defined. It is a serious mistake (and one often made, unfortunately) for a therapist to believe that an unreliable patient, with drug abuse still uncontrolled, can be cured by a job. To induce an employer to take such a patient is a disservice to the employer, the patient, and to all the other patients who do not need to have the prejudices of employers reinforced by bad experiences.

The critical area in which the medical and general counseling staff of the methadone programs must join with the professional services of vocational specialists is in the definition and achievement of job readiness. The clinical staff must prepare the patient for the responsibilities of employment and stand with him in his struggles. The vocational specialists must help evaluate the job readiness of patients without being so demanding of perfection as to exclude the majority of patients from consideration. Everyone must be aware of the strength that a patient derives from pride in identification with a good program and assurance that the staff is concerned with him as a valuable human being (5).

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The Road to Regional Enteritis

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The myth has arisen that the concept of segmental granulomatous diseases of the bowel sprang full-panoplied into being after the individual observation of two cases (1). Actually the conclusions reached were the results of years of prolonged study to which many people contributed. Among those was Dr. William Antopol, whose important contribution we will describe. It is his unknown connection with regional enteritis that occasions the presentation of this memoir dedicated to him.

My own (L.G.) interest in inflammatory granulomatous diseases of the bowel dates from 1925 when I was Dr. Berg's house surgeon, and for the five years following, when I was an adjunct on his ward service and his assistant in private practice. The study of regional enteritis and kindred granulomatous diseases of the bowel were all performed on surgically excised specimens, entirely drawn from cases operated upon by Dr. A. A. Berg. Dr. Berg was an extraordinary individual. The extent of his surgical practice was almost incredible in its day, and would be difficult for one to believe at present. He operated both morning and afternoon six days a week, and not infrequently on Sunday as well. With such a mass of material even relatively uncommon conditions were encountered with a relative degree of frequency. Among them were cases of tumors and masses originally considered to be either neoplasms or tuberculosis which proved to be granulomata for which no known specific cause could be demonstrated. It became obvious that there was a *considerable variety* of such lesions, and a study was begun pathologically to classify the nature of these different entities.

In 1927 (2) a report of a group of cases was published where suspected neoplasms of the colon proved to be sealed-off perforations of the bowel which produced large pericolonic masses without any primary discernible lesion in the bowel. In a number of such cases fish bones were found in the center of large inflammatory masses. The following year, five cases of segmental or tubular stenosis and one case of anular stenosis were reported (3). These were classified generically as "Lesions Secondary to Vascular Impairment of the Bowel." In these cases there was evidence that at a previous operation, extensive vascular insult had occurred, which resulted in a badly compromised but still viable gut. It was remarked at that time that it was impossible to say how large a role this vascular mechanism played as a primary factor in cases where the causal connection was not so clear-cut. And it was also remarked that the end stages of the lesion in which *primary* vascular insufficiency produced necrosis and second-

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ary infection would be difficult to differentiate from a tissue injury primarily causing severe ulcerative damage. It is interesting to note that it was almost twenty years later that this idea began to be studied.

During this same period other types of inflammatory masses were being encountered. It became obvious that further investigations would be too much for any single observer. At this point I approached Dr. Gordon Oppenheimer, later Urologist to The Mount Sinai Hospital. At that time he was working as an assistant in surgical pathology. We agreed to collaborate on the further study of these chronic inflammatory diseases of the bowel which manifested themselves surgically as either tumors or strictures of the bowel. The term "Nonspecific Granulomata of the Intestines" had been introduced in 1923 by two members of The Mount Sinai Hospital Staff, Drs. A. O. Wilensky of the Surgical Staff, and Eli Moschowitz of the Medical Staff. Interestingly enough, at that time, Dr. Moschowitz was Pathologist to the Beth Israel Hospital, a position later held by Dr. Antopol. These two authors had encountered four instances of tumors of the bowel which had been thought to be either neoplasm or tuberculosis. Histologic studies, however, revealed that they were neither of the above nor did they have any other specific cause.

Dr. Oppenheimer and I embarked on an ongoing and retrospective study of all cases where surgical excision had been performed for inflammatory masses either of the large or small intestine. At that time the main purpose of the study was an attempt to furnish additional proof of the nonspecific or idiopathic nature of these specimens; and to classify the various types pathologically and clinically. The study went back to 1920. We searched the files of the general record room, as well as the special classifications section in the Department of Pathology, going back to that date. I recollect very clearly a specimen in a glass case in the museum of the Department of Pathology whose history we finally discovered. It was one of the earliest cases of distal regional enteritis. This specimen, still in its glass case, is reproduced in the article "Non-Specific Granulomata of the Intestines" which appeared in the *Annals of Surgery* (4).

As we progressed in this study, we managed to cull fifty-two cases of nonspecific granulomata of the bowel. Cases of sigmoid diverticulitis were not included because they had already been described at this time. It was emphasized, however, that in addition to the perisigmoidal masses and abscesses due to diverticulitis, a form of the disease occurred characterized mainly by extensive intramural inflammatory changes in the sigmoid. The fifty-two cases mentioned above were classified as follows:

1. Pericolonic granulomata due to sealed-off perforations of the bowel.
2. Intestinal stenosis secondary to vascular lesions of the bowel.
3. Localized hypertrophic, ulcerative stenosis, of the terminal ileum (later termed "regional enteritis").
4. Localized hypertrophic colitis with or without low grade generalized colitis (now called "granulomatous colitis").
5. Simple penetrating ulcers of the colon.
6. Lesions secondary to inflammation of appendages of the bowel.

As we studied these various groups our attention became more and more directed to a group of cases (Group 3 above) which eventually reached twelve in number.* In these specimens, the disease was limited to the distal two to three feet of ileum, ending rather abruptly at the ileocecal valve where the pathological changes were most advanced. Proximally the disease gradually shaded off. Mucosal ulcerations with a tendency to perforate along the mesenteric border were an outstanding feature of the involved segment. The other prominent feature was a very marked hyperplastic acute and chronic inflammatory process involving mainly the submucosa, which became markedly thickened and increased in size. We gave this the name of "Hypertrophic Ulcerative Stenosis of the Terminal Ileum" (the name "regional enteritis" came later).

All of the other types of granulomata included in the classification had been described elsewhere; but, nowhere could we find a description of an idiopathic nonspecific granuloma of the bowel limited to the distal ileum. Therefore, Dr. Oppenheimer and I began to concentrate on the pathological nature of this entity as well as its clinical manifestations. Studies were undertaken to rule out any specific causes such as amebiasis, actinomycosis, syphilis and tuberculosis. All but tuberculosis could be easily ruled out. Therefore bacteriologic studies including inoculations into guinea pigs, rabbits, and chickens were made, as well as the institution of Lowenstein cultures. None of these revealed evidence of tuberculosis. The paraffin blocks which were still available in all these cases continued to resemble tuberculosis. Dr. Oppenheimer and I were both well aware of the fact that our own expertise in the field of microscopy was not sufficient to enable us alone to solve this knotty problem. We therefore reviewed all of these cases with the members of the Department of Pathology—Drs. Gross, Klemperer, Otani, and Antopol. Although tubercle-like structures were present and giant cells could be found, neither caseation nor the presence of tubercle bacilli could ever be demonstrated. At this time Dr. Antopol was engaged in the study of lycopodium granulomata. We discussed the problem of pseudotubercle formation with him. He enthusiastically embraced the problem and was able, by means of special stains, to demonstrate the presence of cells which were in all probability vegetable cells in the vicinity of which giant cells frequently could be demonstrated (4). They were found in all layers of the bowel and in some lymph nodes. We finally developed the hypothesis that the giant cells might be only incidental findings rather than an integral portion of the disease. It is interesting to note that this hypothesis has only recently been revived.

The question of the relationship of this ileal type of granuloma to appendicitis remained to be resolved. Most of the original patients had presented themselves clinically either as chronic intestinal obstruction or enterocolitis, at times the initial symptoms being those of appendicitis. During this same period of time, one of the difficult problems encountered by surgeons were those cases in which intractable fistulae developed following operation for appendicitis. Such fistulae were known to have their inner-opening origins in the terminal ileum. It

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* Dr. Crohn later added two more.

had usually been assumed that these were secondary to the original appendiceal inflammatory changes, compounded by trauma and the presence of drains. We had encountered a number of such cases that had been subjected to a series of previous operations. The appendix was absent and it was difficult to say whether the changes encountered in the bowel were primary or the result of previous operation and trauma. Eventually a case appeared that provided the clue which led to the explanation of this type of clinical manifestation. I recollect very clearly assisting Dr. Sidney Grossman, then the House Surgeon on Dr. Berg's service, in an operation for an appendix abscess. We entered an abscess cavity containing a moderate amount of pus. All efforts to locate the appendix were fruitless. It was decided to drain the abscess and remove the appendix at a later date. The patient continued to drain purulent material. This would stop, the patient would be discharged, and a few weeks later return with a recurrence of fever followed by purulent discharge through the persistent fistulae. At reoperation some months later it was obvious that the fistulae were coming from the terminal ileum. This had all the characteristics of the segmental form of granulomatous disease described above. An ileocolic resection was performed, revealing a normal appendix and a terminal ileum which was the seat of a granulomatous inflammatory disease. The fistulae persisted because of perforation into the mesentery of ulcers in the segment of bowel.

We were now able to classify the clinical manifestations of this form of distal ileitis as:

1. Simulating acute appendicitis.
2. Picture of ulcerative enteritis.
3. Chronic obstruction of the small intestine.
4. Chronic intractable fistulae either to the skin, vagina, bladder, or segments of bowel.

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The Possible Role of Tryptamine in Brain Function and Its Relationship to the Actions of LSD-Like Hallucinogens

W. R. MARTIN, M.D. AND JEWELL W. SLOAN

In a series of studies which have been designed to understand neurohumoral events that are involved in the flexor reflex in the chronic spinal dog, as well as neurohumoral events that are responsible for its facilitation and for the evocation of the stepping reflex, we have studied the actions of LSD-like hallucinogens on the flexor reflex. It had been previously reported that LSD facilitated the flexor reflex (1, 2) in the cat. In the chronic spinal dog, LSD not only facilitated the flexor reflex but evoked the stepping reflex (Fig. 1). The latter phenomenon was of particular interest to us since it is one of the signs of abstinence in morphine-dependent dogs. It was subsequently observed that other LSD-like hallucinogens such as psilocin, mescaline, DOM (2,5-dimethoxy-4-methyl amphetamine) (3), dimethyltryptamine and diethyltryptamine (4) also facilitated the flexor reflex and evoked the stepping reflex in the chronic spinal dog. It was further observed that LSD, psilocin and mescaline increased respiratory rate, pulse rate and pupillary diameter. These are changes that are similar to those observed in man (5–9).

In an attempt to further characterize the nature of the facilitatory actions of the LSD-like hallucinogens, the ability of phenoxybenzamine, chlorpromazine and cyproheptadine to antagonize these effects was studied, and it was found that both cyproheptadine (0.2 mg/kg) and chlorpromazine (5 mg/kg) antagonized the actions of LSD, psilocin and mescaline; whereas, phenoxybenzamine (4 mg/kg) did not. The effect of these antagonists on respiration, pulse rate, and mydriatic actions of the hallucinogens was more complicated. Phenoxybenzamine, chlorpromazine, and cyproheptadine antagonized the mydriatic actions of LSD, psilocin, and mescaline. None of the antagonists antagonized the increase in pulse rate produced by any of the LSD-like hallucinogens to a statistically significant degree, and only cyproheptadine significantly decreased the enhancement of respiratory rate produced by mescaline but not that produced by LSD or psilocin.

Because chlorpromazine and cyproheptadine produced either no depression or only a modest depression of the flexor reflex, it was concluded that their ability to almost completely antagonize the facilitatory effects of the LSD-like hallucinogens could not be due to a physiologic antagonism, that they were acting as antagonists and that the hallucinogens were acting as agonists. Experiments were then initiated to further characterize the properties of the central receptor responsible for the agonistic actions of these hallucinogens to determine if there was a naturally occurring agonist for the receptor in the brain, and to further determine if it played a role in normal and pathologic functioning of the brain. Because of the

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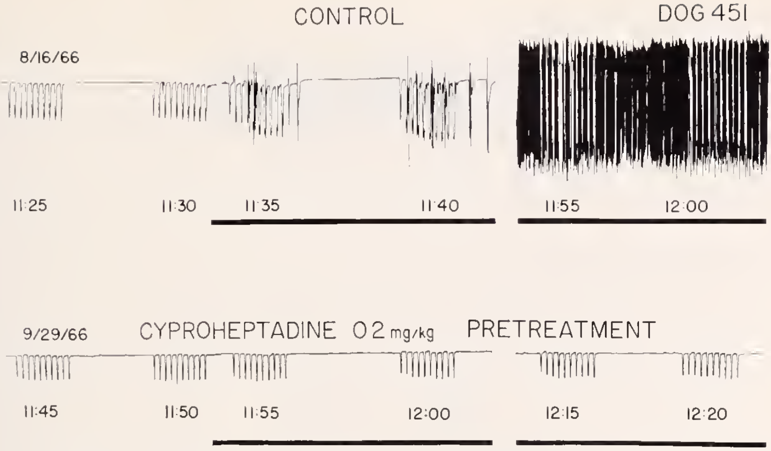

FIG. 1. The effects of LSD on the flexor reflex of the chronic spinal dog and its modification by cyproheptadine. The solid bar indicates the infusion of LSD (0.375 μg/kg/min) which was administered continuously over a 40 min period. The figures below the tracing indicate the times at which the first of a train of toe stimuli were administered. The flexor reflex is recorded as a downward deflection. As can be seen in the upper panel, the amplitude of the flexor reflex was enhanced and spontaneous extension and flexion of the leg as well as fragmentary stepping movements became manifest after only a few min of infusion of LSD. After 20 min of infusion, the stepping reflex had become well developed and continuous, as can be seen in the right hand panel. Further, application of a stimulus to the toe inhibited the stepping reflex and evoked a greatly enhanced flexor reflex. (Reproduced from a paper by Martin and Eades (3) with the permission of *Psychopharmacologia.*)

structural similarities between the LSD-like hallucinogens DMT and tryptamine and because Marley and Vane (10) had previously shown that tryptamine had facilitatory actions on the cat spinal cord, studies were undertaken to compare the actions of tryptamine with those of the LSD-like hallucinogens on reflex activity of the chronic spinal dog.

Tryptamine (0.5 mg/kg/min) facilitates the flexor reflex, evokes stepping movements (Fig. 2) and increases respiratory rate, pulse rate and pupillary diameter. The respiratory and pupillary effects of tryptamine were significantly antagonized by both chlorpromazine and cyproheptadine, as was its ability to evoke the stepping reflex and facilitate the flexor reflex (3). These experiments clearly demonstrated that actions of tryptamine in the dog were similar to those of the LSD-like hallucinogens.

The actions of tryptamine on the spinal cord of the chronic spinal dog have been clearly distinguished from those of methoxamine, an alpha adrenergic agonist, amphetamine, oxotremorine, physostigmine and 5-hydroxytryptamine through the use of antagonists and 5-hydroxtryptophan. The actions of methoxamine and amphetamine are antagonized by phenoxybenzamine and chlorpromazine but only to a slight degree by cyproheptadine. The actions of oxotremorine and physostigmine are antagonized by atropine but not phenoxybenzamine; whereas, the actions of tryptamine are antagonized by chlorpromazine and cyproheptadine but not by phenoxybenzamine. In contrast to the actions of tryptamine, those of 5-OH-tryptophan are not antagonized by cyproheptadine (3).

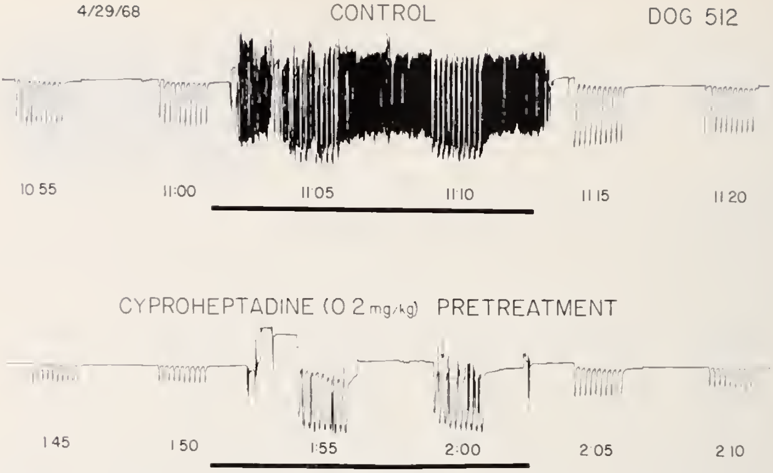

FIG. 2. The effects of tryptamine (0.5 mg/kg min) on the flexor reflex of the chronic spinal dog and the antagonism of its effects by cyproheptadine. The solid bar indicates the period of tryptamine infusion. The flexor reflex is recorded as a downward deflection. The figures beneath the tracing indicate the time at which the first of a train of toe stimuli were administered. The stepping reflex became manifest after only a minute or two of tryptamine infusion, and subsided within a minute after the termination of the infusion. The flexor reflex was clearly facilitated at the time of the first stimulus train after the infusion was terminated. (Reproduced from a paper by Martin and Eades (3) with the permission of *Psychopharmacologia.*)

When tryptamine is infused intravenously in man, its effects are similar to those seen in the dog and to LSD in man (11). It dilates pupils, increases blood pressure, enhances the patellar reflex and produces a variety of changes in perception and sensation, particularly auditory and visual changes.

These lines of evidence would indicate that LSD and tryptamine share a common mode of action. When LSD was administered chronically to the spinal dog, tolerance developed to its ability to facilitate the flexor reflex, evoke the stepping reflex and increase pulse rate, respiratory rate and pupillary diameter (Table I).

TABLE I

Direct Tolerance to LSD in Dogs Receiving LSD (15 µg/kg) Twice Daily Subcutaneously

	Control	1st week	2nd week
Pulse (Beats-min)	5012	$294^{.025}$	$783^{.025}$
Respiration (Breaths-min)	4589	$1874^{.2}$	$1078^{.2}$
Pupils (mm-min)	231	$-155^{.025}$	$-229^{.001}$
Flexor reflex (mm-min)	411	$365^{.10}$	$339^{.05}$

The values for pulse rate, respiratory rate and pupillary diameter represent the group mean of the areas under the time action curve for the 160 min observation period after the beginning of the LSD infusion. Superscripts indicate the level of significance of differences between the control response and the responses when the dogs had received LSD chronically (15 µg/kg b.i.d.) for 1 and 2 weeks, using a paired "t" test. (Reproduced from a paper by Martin and Eades (12) with the permission of *Psychopharmacologia.*)

When dogs are tolerant to LSD, they are cross-tolerant to these actions of tryptamine (12).

These data support the hypothesis that LSD and related hallucinogens exert many of their effects by serving as agonists of a tryptamine receptor. More recently, we have attempted to determine if tryptamine plays a role in the normal functioning of the brain and whether it could, if present in excessively high levels, give rise to disturbed brain functioning. To partially answer the first question, we have over the last three to four years developed methods to determine if tryptamine is present in the brain and to measure its concentration. To these ends, highly selective extraction procedures, as well as modifications of spectrophotofluorometric (SPF) methods for the measurement of brain tryptamine and gas liquid chromatographic (GLC) and thin layer chromatographic (TLC) methods for its identification, have been developed (13, 14).

Studies of the presence of tryptamine in the rat brain have yielded equivocal results. Using SPF methods, brain levels of tryptamine were estimated to be approximately 20 ng/g; however, in these studies the SPF scans were not typical for norharman, the fluorescent compound to which tryptamine is converted for its estimation. Further, we could not demonstrate the presence of tryptamine in the brain using GLC and TLC, which should have had sufficient sensitivity to identify tryptamine had it been present. However, more recently Saavedra and Axelrod (15, 16) using an enzymatic procedure have identified tryptamine in the rat brain.

Tryptamine was clearly identified in the steer brain and was present in its highest concentrations in the basal ganglion, thalamus and hypothalamus, and mesencephalon (14). Tryptamine was present, however, throughout the brain and its identity was confirmed using GLC and TLC.

Tryptamine has also been identified in the brain and spinal cord of the dog and its presence has been confirmed with GLC methods (Table II).

Of particular interest is the fact that the dog spinal cord has relatively high levels of tryptamine. Pretreatment with the monoamine oxidase inhibitor isocarboxazid increases brain levels of tryptamine by a factor of 3. It was in the dog that we also first identified, using GLC methods, melatonin in all major areas of the brain.

More recently, studies have been made of brain levels of tryptamine in the chronic spinal dog. Tryptamine was found in the lumbosacral cord, below the level of transection, in amounts not different from those found in the intact dog. These findings would indicate that the spinal cord tryptamine has, at least in part, a different origin from the spinal cord serotonin and norepinephrine for spinal cord levels of these neurohumors are markedly depleted following transection of the spinal cord and degeneration of descending pathways.

We were also able to demonstrate the presence of tryptamine in the human brain, an experiment that would not have been done except through the interest and the help of Dr. William Antopol (Table III). The levels of tryptamine are higher in the human brain than those found in either the bovine or dog brain.

TABLE II

TRYPTAMINE — DOG BRAIN

AREA	UNTREATED DOG		ISOCARBOXAZID TREATED	
	SPF ng/g ± S.E.(n)	GLC	SPF ng/g ± S.E.(n)	GLC
CEREBRAL CORTEX	28 ± 5 (10)	PRESENT	78 ± 14 (14)	PRESENT
BASAL GANGLIA	88 ± 23 (5)	PRESENT	182 ± 43 (6)	PRESENT
THALAMUS + HYPOTHALAMUS	49 ± 18 (3)	PRESENT	83 ± 48 (3)	PRESENT
MESENCEPHALON	15 ± 9 (3)	PRESENT	291 ± 105 (3)	PRESENT
PONS + MEDULLA	2 (1)	?	579 ± 257 (3)	PRESENT
CEREBELLUM	40 ± 9 (6)	PRESENT	76 ± 18 (6)	PRESENT
CERVICAL AND THORACIC CORD	49 ± 9 (6)	PRESENT	146 ± 37 (7)	PRESENT
LUMBAR AND SACRAL CORD	47 ± 20 (4)	PRESENT	204 ± 61 (4)	PRESENT

Each SPF value, corrected for standard recovery, represents the mean ± one standard error. The number of specimens analyzed is shown in parenthesis. (Reproduced from a paper by Martin et al (14) with the permission of *Psychopharmacologia.*)

TABLE III

TRYPTAMINE
DETERMINATIONS IN ONE HUMAN BRAIN

AREA	SPF ng/g (RANGE) (n)	GLC
CEREBRAL CORTEX	172 ± 46* (0-410) (9)	PRESENT
BASAL GANGLION	150 (105-194)(2)	PRESENT
THALAMUS + HYPOTHALAMUS		PRESENT
CEREBELLUM	389 (375-403)(2)	PRESENT

* = ± SE

Each SPF value represents the mean (ng/g) ± S.E. or range (in parenthesis). The number of specimens is also indicated in parenthesis. (Reproduced from a paper by Martin et al (14) with the permission of *Psychopharmacologia.*)

Although experiments on regional perfusions of the dog brain are still in progress, we have demonstrated that tryptamine is released from the brain in this species.

Of great interest to us is the question whether tryptamine could play a role in the pathologic functioning of the brain. Our own data in man suggest that it could, for the infusion of relatively large quantities of tryptamine produces changes similar to those produced by LSD, including perceptual distortions. Further, Brune and Himwich (17, 18) and Herkert and Keup (19) found that exacerbation of the schizophrenic process was associated with an increase in the excretion of tryptamine in the urine. Administration of tryptophan, the precursor of tryptamine, in schizophrenic patients receiving large doses of iproniazid was associated with mood elevation and enhancement of the patellar reflex (20), changes seen after modest doses of LSD in nontolerant subjects (6). Finally, platelet monoamine oxidase activity has been reported to be low in schizophrenic patients (21), and less in schizophrenic twins than in their nonpsychotic cotwins (22). Monoamine oxidase is the enzyme responsible for the inactivation of tryptamine. These findings are consistent with the hypothesis that tryptamine may play a role in the normally functioning brain and when present in excessively high quantities may be responsible for pathologic functioning.

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Hyperplasia and Neoplasia of the Thyroid in Marine Fishes

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Introduction

Except for cartilaginous fishes, and certain teleosts (e.g., lungfishes, parrotfishes), the fish thyroid is an unencapsulated structure usually found as minute follicles in the loose connective tissue of the subpharyngeal and parapharyngeal areas in the vicinity of the ventral aorta and bulbous arteriosus, between the first and second gill arches (1–3). The number and size of follicles, height of epithelial cells, and the amount of colloid differ considerably under the same or different physiological conditions between species or individuals of the same species (4, 5). The presence of ectopic follicles in the head kidney, heart, choroid gland and other structures is not unusual in some fishes (6, 7).

Thyroid "tumors" were first reported by Bonnet in 1883 in lake trout (*Salmo lacustris*) in a German hatchery (8). Since then similar abnormal growths in hatchery-reared trout and salmon were reported all over the world, and in a variety of other captive and feral freshwater and marine fishes (9–15). (See Table I for current summary of thyroid tumors in marine fishes.)

The spontaneous development of goiters and thyroid tumors in strictly marine (stenohaline) fishes poses some questions as to etiology since these fish live in an environment, even under captive conditions, relatively rich in iodine and feed on, or are fed, fish, clams, shrimps and other organisms with high iodine content. The present report deals with the spontaneous development of hyperplastic and neoplastic thyroids in a number of individuals of two highly active stenohaline species kept in captivity at the New York Aquarium, namely, *Plotosus anguillaris* (Pacific coral catfish) and *Opistognathus aurifrons* (Atlantic yellowhead jawfish) (Figs. 1 and 2).

Observations

Seven of fifty-one coral catfish and six of forty-five yellowhead jawfish were observed with thyroid swelling protruding from one or both branchial cavities (Fig. 3). The incidence is given in Table II. The affected fish showed some degree of emaciation and labored respiration. No pronounced exophthalmia was evident. As is usual in such cases, the apparent anoxia is due to extensive destruction of muscle, bone, and cartilage of the water-pumping mechanism by the proliferating tissue, and by reduced blood flow to and from the gill capillaries caused by pressure of the growth on the ventral aorta and/or on the afferent and efferent branchial blood vessels. In extreme instances, gill lamellae

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TABLE I

Spontaneous Thyroid Tumors in Marine Fishes

Species	No. Specimens	Locality
Class: Chondrichthyes		
Order: Heterodontiformes		
Family: Heterodonidae		
Heterodontus francisci Horn Shark	1	Shedd Aquarium, Chicago* (Present Paper)
Order: Squaliformes		
Family: Orectolobidae		
Ginglymostoma cirratum Nurse Shark	9	Shedd Aquarium* (Present Paper)
	1	N.Y. Aquarium
Family: Carcharhinidae*		
Negaprion brevirostris Lemon Shark	1	Shedd Aquarium* (Present Paper)
Triakis semifasciata California Leopard Shark	1	Shedd Aquarium* (Present Paper)
Family: Squalidae		
Squalus suckleyi† California Dogfish	1	Cameron & Vincent, 1915 (21)
Family: Rajidae		
Raja laevis (*R. stabuliformis*) Barndoor Skate	1	Burwash, 1929 (22)
Class: Osteichthyes		
Order: Siluriformes		
Family: Plotosidae		
Plotosus anguillaris Coral Catfish	7	N.Y. Aquarium (Present Paper)
Order: Atheriniformes		
Family: Cyprinodonitidae		
Fundulus heteroclitus Mummichog	5	N.Y. Aquarium Nigrelli, 1952 (10)
Cyprinodon variegatus Sheepshead Minnow	10	N.Y. Aquarium Nigrelli, 1952 (10)
Order: Perciformes		
Family: Serranidae		
Serranus scriba	1	Naples Aquarium
Serranus cabrilla Cabrilla	5	Naples Aquarium Marsh & Vonwiller, 1916 (23)
Family: Echeneidae		
Echenesis naucrates Sharksucker	16	Fairmount Park Aquarium, Philadelphia Schlumberger & Lucké, 1948 (9) Schlumberger, 1955 (24)
Family: Carangidae		
Trachinotus carolinus Florida Pompano	1	Harshbarger, 1971–1972 (13, 14)
Family: Sparidae		
Box vulgaris (Box boops)	1	Feral Fish (England) Johnstone, 1924 (25)

TABLE I—*Continued*

Species	No. Specimens	Locality
Family: Chaetodontidae		
Holocanthus ciliaris (*Angelichthys isabelita*)	1	N.Y. Aquarium Nigrelli, 1952 (10)
Blue Angelfish		
Family: Opistognathidae		
Opistognathus aurifrons	6	N.Y. Aquarium (Present Paper)
Yellowhead Jawfish		
Family: Trichiuridae		
Trichirius lepturus	1	Feral Fish (Portugal) Pinto, 1958 (21)
Atlantic Cutlassfish		
Family: Scorpaenidae		
Sebastes crameri	1	Harshbarger, 1972 (14)
Darkblotched rockfish		

* Observations by the senior author; characteristic enlargement present in "throat" region.

† *Squalus acanthias*(?).

may be destroyed by the proliferating tissue. Since the gills also function in excretion of nitrogenous waste, the extensive tubular degeneration (Fig. 15) of the kidney in fish with thyroid tumors must be considered as a contributing cause of death, in addition to the metabolic disturbances inherent in these thyroid changes.

Microscopically, the normal gland in coral catfish is typical of most teleosts, consisting of relatively few follicles scattered in the loose connective tissue of the subpharyngeal region (Fig. 4). The gland in the yellow jawfish, however, is a bilobed structure surrounded by a thin capsule, reminiscent of the gland in higher vertebrates (Fig. 5).

The thyroid growths in yellowhead jawfish range from simple hyperplasia to extensive proliferation, filling the entire subpharyngeal and parapharyngeal space. The growths are characterized as primary and secondary hyperplasia, with follicles lined with cuboidal and/or columnar epithelial cells but with very little colloid. There is some connective tissue development in the regions where secondary nodules are formed (Figs. 6–9).

The growths in the coral catfish ranged from varying degrees of hyperplasia to the development of interacinous adenoma and carcinoma, in one instance. Microscopically, the goiters were typical, with follicles lined with flattened or cuboid epithelial cells and filled with colloid (Fig. 10). In extreme hyperplasia, a few macrofollicles and numerous microfollicles, with or without colloid, are present (Fig. 11). Numerous cells in mitosis were seen in the interacinous areas (Fig. 12). In some instances the extensive hyperplasia is characterized by papillae-like formations (Fig. 13) that are distinguished by the development of small follicles enclosed in their stroma (Fig. 14). This fish was also of interest for the development of giant cells (Fig. 16) in the pharyngeal muscle adjacent to the proliferating growth, and by extensive tubular degeneration of the kidney (Fig. 15). Figs. 17 through 20 show the development of an interacinous adenoma and carcinoma, respectively. Microscopically, the adenoma is surrounded by a thin capsule and the cells, with bizarre nuclei, are highly vacuolated. A few mitotic figures were seen. The carcinoma was massive, involving

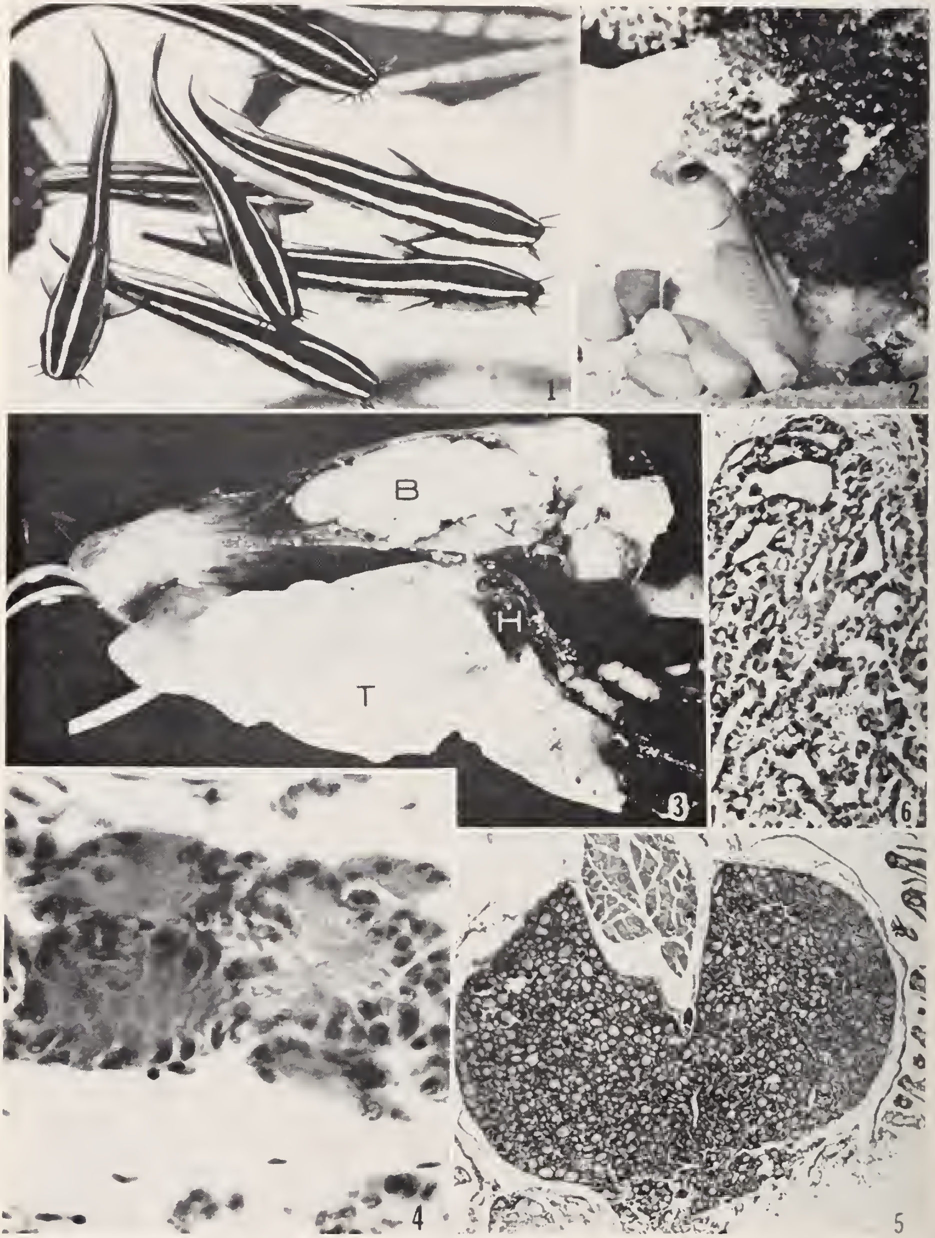

PLATE I

Figs. 1–6.

TABLE II

Year	No. Fish	No. Tumors
Spontaneous Thyroid Tumors in Coral Catfish		
1966	2	0
1968	12	3
1969	7	4
1970	15	0
1971	14	0
1972	1	0
Total	51	7 (13.7%)
Spontaneous Thyroid Tumors in Yellowhead Jawfish		
1963	10	2
1964	2	2
1966	10	0
1967	9	0
1968	2	0
1969	2	1
1970	3	0
1971	3	0
1972	4	1
Total	45	6 (13.3%)

all the structures of the pharyngeal region, including the gills. The growth was characterized by the presence of unorganized cells but with a few microfollicles still visible. Although extensive hemorrhage was present, there was no evidence of invasion of the blood vessels.

PLATE I

FIG. 1. Pacific coral catfish, *Plotosus anguillaris*. Certain individuals from such small, tightly forming, highly active schools frequently develop hyperplasia or neoplasia of the thyroid. ¾ natural size. Photo by W. Lerchenfeld, New York Zoological Society.

FIG. 2. An Atlantic yellowhead jawfish, *Opistognathus aurifrons*, in its burrow. The fish, which is susceptible to thyroid hyperplasia, constantly keeps cleaning its burrow by picking the pebbles that fall in with its mouth and spitting them out around the hole. This species has strong territorial behavior. ¾ natural size. Photo by W. Lerchenfeld, New York Zoological Society.

FIG. 3. A sagittal cut made along the midline showing extent of massive thyroid tumor in coral catfish. T = tumor mass; H = heart; B = brain. Section of this thyroid is shown in Fig. 17. × 5. Photo by W. Lerchenfeld, New York Zoological Society.

FIG. 4. Normal coral catfish thyroid follicles, lined with flattened epithelial cells, with and without colloid, in the loose connective tissue in vicinity of the ventral aorta. Hematoxylin-eosin. × 550.

FIG. 5. Hypertrophied thyroid gland of yellowhead jawfish surrounded by a thin capsule. In normal fish the thyroid is smaller, and reminiscent of bilobed structure seen in sharks and a few teleosts, such as parrotfishes, and in higher vertebrates. The follicles, lined with flattened epithelial cells, are more or less uniform in size and evenly filled with colloid. Hematoxylin-eosin. × 275.

FIG. 6. Edge of proliferating hyperplastic thyroid in yellowhead jawfish, consisting mainly of small follicles with flattened or cuboidal epithelial cells and mostly devoid of colloid. Hematoxylin-eosin. × 550.

Discussion

Interest in the spontaneous development of hyperplasia and neoplasia of the thyroid in fishes reached a high point with the publication of the controversial treatise on "Carcinoma of the Thyroid in Salmonoid Fishes" (16). A reexami-

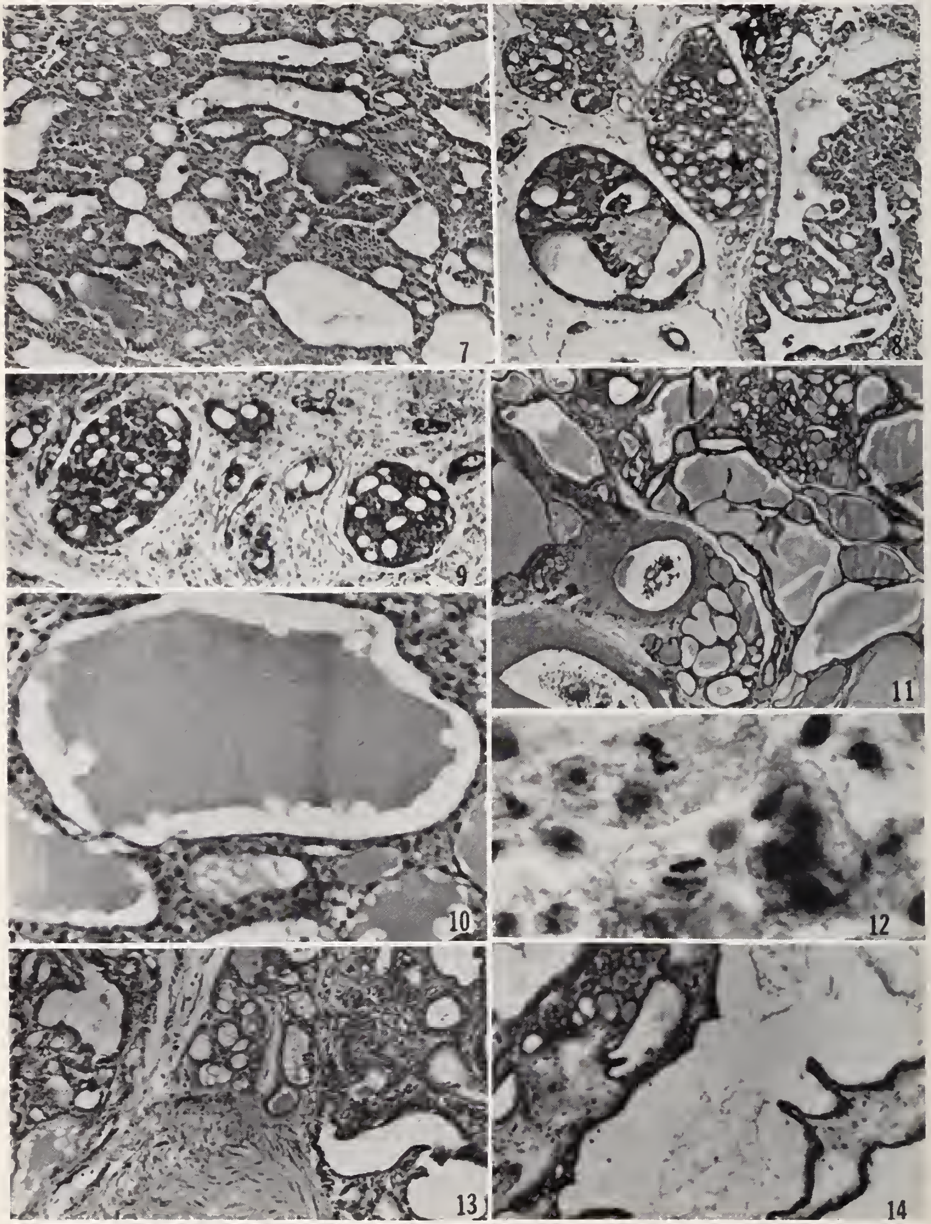

PLATE II
Figs. 7–14.

nation of the histological descriptions and the photographs of this extensive report had convinced many of the early thyroidologists, including Marine (17), that the tumors were examples of goiters and colloid storage, which could be prevented or ameliorated by the addition of iodine to the water or feed, a regimen that is standard practice as a prophylaxis in trout and salmon hatcheries all over the world.

However, in the light of present-day interpretations, no sharp distinction of epithelial hyperplasia, benign and malignant tumors of the thyroid is possible (18). This is especially true for fishes in which the unencapsulated follicles, such as seen in coral catfish, are highly reactive to a variety of environmental and physiological stimuli, and capable, especially in susceptible species, of relatively rapid and extensive proliferation, and extensive destruction of all invaded tissues. Thus, it is not surprising that many of the hundreds of abnormal growths of the thyroid examined by Gaylord and Marsh, and their collaborators, could be classed as neoplasms, and some of them were definitely carcinomas with metastases.

Thyroid hyperplasias and tumors occur more frequently in freshwater fishes than in marine species (9, 15), and certain species, especially in the families Salmonidae and Poeciliidae, are especially prone, suggesting a genetic basis for susceptibility (19). This apparent susceptibility is indicated by the striking hyperplastic responses of the thyroid in this group of fishes to thiourea, thiouracil and other antithyroid drugs, when compared to other freshwater and brackish (euryhaline) fishes similarly treated. In some instances, prolonged treatment with such drugs will induce adenomatous-like tumors (4, 20). The thyroids of strictly marine fishes, with few exceptions, are relatively resistant to such drugs (Table III).

The spontaneous development of hyperplasia and neoplasia of the thyroid in stenohaline fishes kept in captivity suggests that factors other than iodine deficiency may be responsible. The senior author (10), and others (4), have suggested that the thyroid changes may be due to environmental factors such as low oxygen tension, low light intensity, and changes in salinity and/or abnormal calcium-magnesium ratios. It is further suggested that stimulation of the thyroid, especially in fishes with relatively higher requirement for iodine, in

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PLATE II

FIG. 7. Hyperplastic thyroid in yellowhead jawfish; follicles lined with columnar cells. Most follicles lack colloid. Hematoxylin-eosin. × 550.

FIG. 8. Section of hyperplastic thyroid in yellowhead jawfish showing primary and secondary nodules. Hematoxylin-eosin. × 275.

FIG. 9. Secondary hyperplastic nodules in yellowhead jawfish, in adjacent connective tissue, each surrounded by a delicate capsule. Hematoxylin-eosin. × 275.

FIG. 10. Example of nodular goiter, with flat epithelium and abundant colloid in coral catfish. Hematoxylin-eosin. × 275.

FIG. 11. Hyperplastic growth around the ventral aorta, coral catfish. Hematoxylin-eosin. × 275.

FIG. 12. Mitotic cells in the interacinous elements of the growth shown in Fig. 10, coral catfish. Hematoxylin-eosin. × 550.

FIG. 13. Example of secondary hyperplasia, bordering on a cystadenoma, in coral catfish. Note infiltration into walls of conus arteriosus. Hematoxylin-eosin. × 275.

FIG. 14. Note small follicles in stroma, coral catfish. Hematoxylin-eosin. × 500.

closed circulating systems may be due to accumulation of urea, ammonia and other potential goitrogens, or to chemicals in the environment that may interfere with enzymes involved in the major biosynthetic reaction necessary for the secretion of thyroxine. Further, Pickford (4) suggested that an analysis of the

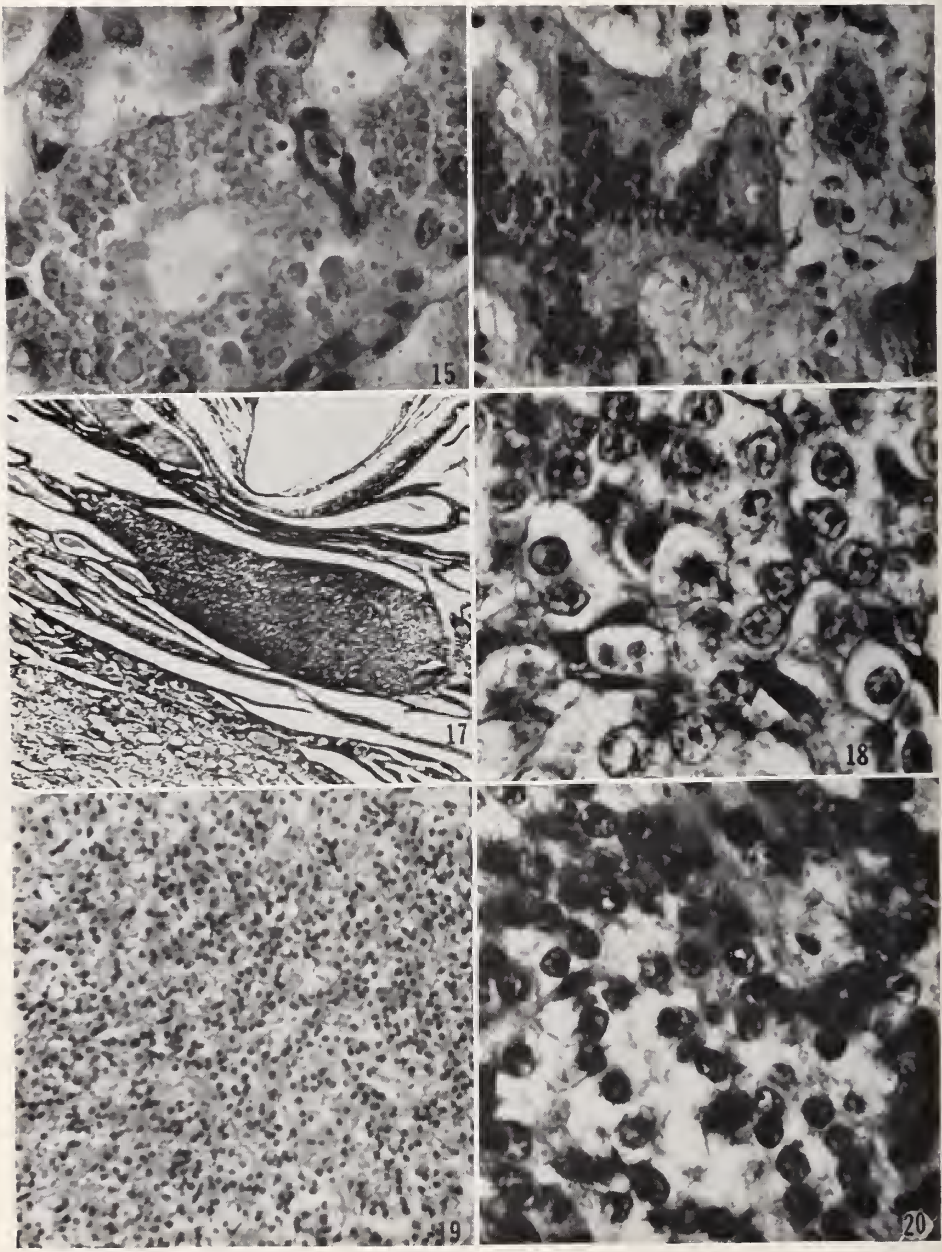

PLATE III

Figs. 15–20.

TABLE III

Hyperplastic Responses of Thyroid Tissue of Marine Fishes to Antithyroid Drugs (Thiourea, Thiouracil, etc.) (Modified after Pickford, 1957)

	Response
Marine Species	
Elasmobranchs	
Mustelus mustelus (*M. laevis*)	++¹
Teleosts (Stenohaline)	
Box salpa	−
Callionymus lyra	+
Conger conger	+
Dentex dentex (*D. vulgaris*)	+
Gobius cobitis (*G. capito*)	+
Mugil auratus	+
Sparids (*Spariosoma* sp.; *Pseudascaris* sp.; *Scarus* sp.)	++
(Euryhaline)	
Fundulus heteroclitus	++
Fresh-Water Species	
Lebistes reticulatis	++
Xiphophorus (*Platypoecilus*) *maculatus*	++
Xiphophorus helleri	++
Salmo trutta (fry)	++
Oncorhynchis keta (alevins)	++

− = Little or no effect.
+ = Moderate stimulation.
++ = Marked hyperplasia.
¹ = With hyperemia.

data on thyroid hyperplasia tends to support the hypothesis that thyrotropins may be low in marine fishes. The possible role of the pituitary in the development of hyperplastic thyroids in marine fishes is suggested by our observations that such fish in captivity do not develop mature gonads, while comparable fish in their natural environment are sexually mature.

Irrespective of the stimulus that may be responsible for the thyroid tumors in the coral catfish and yellowhead jawfish, it is very difficult to sharply define the pathologic nature of the growths. Even though most of the tumors are representative of hyperplastic responses, the lesions created are nevertheless malignant in the sense that the growths are capable of extensive proliferation, causing destruction of all surrounding tissue, including such vital organs as the gills.

PLATE III

Fig. 15. Tubular degeneration in the kidney present in coral catfish with thyroid hyperplasia shown in Figs. 13 and 14. Hematoxylin-eosin. × 2750.

Fig. 16. Giant cell formation in the muscle of the branchial structure in coral catfish with tumor shown in Figs. 13 and 14. Hematoxylin-eosin. × 2750.

Fig. 17. Interacinous adenoma, coral catfish. Hematoxylin-eosin. × 275.

Fig. 18. Cellular details in the solid portion of the tumors shown in Fig. 17. Hematoxylin-eosin. × 550.

Fig. 19. Carcinoma with some evidence of acini, coral catfish. There was considerable hemorrhage present. Hematoxylin-eosin. × 275.

Fig. 20. Cellular details of carcinoma shown in Fig. 19. Hematoxylin-eosin. × 2750.

Summary

Seven of fifty-one coral catfish (*Plotosus anguillaris*) and six of forty-five yellowhead jawfish (*Opistognathus aurifrons*) were found with thyroid abnormalities that ranged from simple goiters to carcinomas. Coral catfish, with unencapsulated thyroid, showed more dramatic hyperplastic changes when compared to the hyperplasia in yellowhead jawfish that possess an encapsulated thyroid.

The possible causes of the hyperplasia and tumors in these stenohaline fishes are discussed.

Acknowledgment

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The Sydenham Society (1843–1857): Rise and Fall

WILLIAM B. OBER, M.D.*

One of the great pleasures in working with William Antopol was that we shared an interest in medical history and collecting medical books. At his death, he left an extensive collection, chiefly of nineteenth century imprints. Among them were some volumes of the Sydenham Society's publications, and these he prized. A few months before he died, I returned from a trip to Australia where I had the good fortune to see the entire set of books published both by the Sydenham Society itself and its successor, the New Sydenham Society. They were arranged attractively in the Rare Book Room of the University of Melbourne's medical school library, an impressive array, reflecting both an important aspect of British medical education in the nineteenth century and the taste of Professor Kenneth F. Russell in displaying the Rare Book Room's holdings. I described this in detail to Dr. Antopol, and we procured a list of the titles, spending the better part of an afternoon discussing them. It was one of the last long conversations we had; thus, a résumé of the Sydenham Society's accomplishment seems a suitable subject for this memorial issue.

This account of the Sydenham Society's operations has been pieced together from the published reports of its annual general meetings†, other "ephemera" in the Library of the New York Academy of Medicine, and from contemporary accounts in medical journals. The importance of its work rests upon the 30 titles in 40 volumes which it published between 1843 and 1857, many of them now classics, which formed the nucleus for medical reading by English-speaking physicians for several generations. Even after the Sydenham Society itself went out of business, its goals were so essential to the intellectual needs of the profession that the New Sydenham Society was promptly founded, profiting from some of its predecessor's mistakes, but nonetheless patterning its operations upon it to a considerable degree. The successor organization managed to publish 74 titles in 185 volumes between 1859 and 1907.

The Sydenham Society was founded in 1843 on the plan of the Camden Society (1838) which published works of antiquarian and historical interest and the Parker Society (1841) devoted to publishing writings of the fathers of the English Reformed Church. The former was taken over by the Royal Historical Society later in the century, but the latter succumbed in 1854. More

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† I do not have a complete file of these reports. The Library of the New York Academy of Medicine has the reports for 1846, 1848, 1850, 1852, 1853, 1854, and 1856. I am indebted to Dr. Peter D. Olch of the National Library of Medicine for the reports for 1844, 1849, 1851, and 1857. I have not been able to locate reports for 1845, 1847, and 1855.

demand existed for medical than theological texts; the Sydenham Society survived until 1857, and the New Sydenham Society arose phoenix-like the same year, to publish its first books two years later.

The stated purpose of the Sydenham Society was to meet "certain acknowledged deficiencies in existing means for diffusing medical literature, which are not likely to be supplied by the efforts of individuals," and it proposed a remedy by publishing six categories of books:

> 1. Reprints of standard English works, which are rare or expensive; 2. Miscellaneous selections from the ancient and the earlier modern authors, reprinted or translated; 3. Digests of the works of old and voluminous authors, British and foreign . . .; 4. Translations of the Greek and Latin medical authors, and of works in the Arabic and other Eastern tongues, accompanied, when it is thought desirable, by the original text; 5. Translations of recent foreign works of merit; 6. Original works of merit, which might prove valuable as books of reference, but which would not otherwise be published, from the slender chance of their meeting with a remunerative sale.

Membership in the Society cost one guinea a year and entitled the subscriber to all the volumes published that year, three or four as the case might be. A system of Honorary Local Secretaries was developed to canvass for members and to supervise distribution. By 1844, 112 doctors were acting as local agents, including three in the United States. The Society's *modus operandi* entitles it to be called the first of the medical book clubs, and the content of its books qualifies it for priority as a medical reprint house. Policy was entirely controlled by members of the medical profession, and the Society's aims were educational rather than commercial.

During the first half of the nineteenth century British medicine underwent a transition, "the change from the old medicine of history-taking and elaborate treatment to the medicine of physical examination and the attempt to apply scientific therapy" (1). All periods of history are transitional, but some are more transitional than others. It would be facile to delimit the chronology of this period by the two administrative landmarks, the Apothecaries Act of 1815 and the Medical Act of 1858 under which the General Medical Council was formed, which changed the structure of British medicine. But the remodelling was gradual, part of a slowly evolving development in all biological sciences, and the parliamentary acts are to be taken as signposts of the way in which physicians were being educated and of their changing ideas about the practice of medicine.

Much of the impetus for this gradual shift in values stemmed from a brilliant constellation of French doctors at the turn of the century. The work of Bichat, Corvisart, Pinel, Magendie, and Laënnec made Paris the medical capital of the world during the first third of the century, and their monographs were translated into English, widely read and influential. British physicians kept step, and a number of monographs reflected the new ideas, *viz.:* Sir Astley Cooper's handsome texts, *The Anatomy and Surgical Treatment of Abdominal Hernia* (1804–07) and *Illustrations of Diseases of the Breast* (1829), James Parkinson's *An Essay on the Shaking Palsy* (1817), Sir Benjamin Brodie's *Pathologi-*

cal and Surgical Observations on Diseases of the Joints (1818) and his *Lectures on the Diseases of the Urinary Organs* (1832). In his *Reports of Medical Cases* (1827–31) and subsequent articles Richard Bright had laid a foundation for correlating history-taking and physical examination with laboratory investigation, postmortem examination, and an approach to rational therapy. James Hope's *Diseases of the Heart and Great Vessels* (1831) was a work of considerable stature in cardiology.

British schools of anatomy had always been strong, and the tradition of John and William Hunter had been continued by John and Sir Charles Bell whose finely illustrated dissections were published at the turn of the century. Quain's *Elements of Descriptive and Practical Anatomy* was first published in 1828, went through ten more editions in eight decades, and served as an instruction manual for generation after generation of British medical student. But Gray's *Anatomy, Descriptive and Surgical* was not published until 1858, the year of the Medical Act. The increasing importance of morbid anatomy was taught and made felt in the museums structured by Edward Stanley at St. Bartholomew's (1825 ff.), Thomas Hodgkin and other clinician-pathologists at Guy's (1825 ff.), and by Sir James Paget at St. Bartholomew's (1837–43). Cruveilhier's *Anatomie pathologique du corps humain* (1829–42) could be matched by Sir Robert Carswell's *Illustrations of the Elementary Forms of Disease* (1837). In the broad area of internal medicine the first edition of Thomas Watson's *Lectures on the Principles and Practice of Physic* appeared in 1843, the year the Sydenham Society was founded. Periodical literature also expanded during the period. The *Lancet* was founded in 1823 and *Guy's Hospital Reports* first appeared in 1836; other new journals appeared, flourished for a while, then disappeared. The pattern emerges that with widening of medicine's horizons British physicians and medical students required a larger number of books and a wider range of subjects treated.

It is not entirely clear who first conceived the idea of the Sydenham Society, but once the scheme was formulated, the needs of the profession were so apparent that there was no difficulty in securing many of the leading physicians in England and Scotland to serve as the Society's officers or on its Council. Familiar names on the roster include Sir Henry Acland, Thomas Addison, Sir Benjamin Brodie, Sir James Clark, Sir Henry Holland, Thomas Hodgkin, Jonathan Hutchinson, Thomas Watson, and many prominent figures of the day who occupied either high administrative positions or academic chairs. The presidency of the Society rotated annually and was filled by a man of considerable prominence. Also on the masthead were 16 vice-presidents and 24 council members, men of high standing, most of them based in London, but a few judiciously chosen for geographical distribution. James Risdon Bennett served as secretary and Benjamin Guy Babington as treasurer of the Society throughout its existence. Doubtless, these two competent physicians managed most of the regular conduct of the Society's affairs. They were among the first to help organize the Society and may well have been the early originators of the scheme.

The purpose for which the Society was designed and the quality of its leadership were such that the *Lancet*, not given to inflated encomiums on Establishment figures, bestowed its blessing on the new organization (2):

> We augur well of this society, not merely on account of the laudable nature of its objects, but also because it does not appear to have been got up by any *clique*, but to offer a common ground on which gentlemen of various orders in the profession, and of the most opposite opinions in medical politics, have agreed to meet for the promotion of the case of good letters. Nothing can be more calculated to weaken the influence of those silly distinctions of grade which now tend to disorganise the profession, and to impress on the minds of its members that literature and science are a republic into which no heraldry can be admitted but that founded on intellectual superiority.

The *Lancet* may have seized the occasion to thump its democratic drum, but the Society's objectives were laudable. The need for medical books was so great that it is no surprise to read in the first annual report (1844) that 1700 doctors had enrolled and that the number continued to increase. At the time there were about 18,000 licensed medical practitioners in Great Britain. To attract 10 per cent of any profession into a book club is no small achievement. However, the *Lancet* sang a different tune some 14 years later.

Given this background, it is convenient to examine the merchandise. Just what books did the Society publish? Its prospectus suggests a strong emphasis on translation, and of the 30 published titles, only 4 were works originally written in English. These are: 1. *The Works of William Hewson* (1846). Hewson (1739–74) was William Hunter's brilliant young associate whose career was cut short by sepsis from a wound sustained during a dissection. His work entitles him to be called the first British hematologist, and the volume includes his essays on the properties of blood, the lymphatic system, and the red blood cell; 2. *Essays on Puerperal Fever, &c.* (1850). Edited by Fleetwood Churchill, this is a compilation of nine essays on childbed fever written between 1768 and 1795. The topic was timely. Churchill's rambling introduction concluded that puerperal sepsis was contagious and could be carried by an intermediate party, e.g., a midwife or accoucheur, and his opinion contradicted that of the assembled authorities cited from the previous century; 3. *Anatomia uteri humani gravidi tabulis illustrata* (1851), a folio-size reproduction of William Hunter's splendid engravings of the pregnant human womb, originally published in 1774, a monument in British anatomical studies. Even in 1851 the original elephant folio with its 34 plates was a collector's item, and the reprint fulfilled the prospectus's promise of "standard English works which are rare or expensive"; 4. *Annals of Influenza, or Epidemic Catarrhal Fever in Great Britain from 1510 to 1837* (1852). Edited by Theophilus Thompson, this is a convenient source book of epidemiology, containing first hand observations by Willis (1658), Sydenham (1675), Arbuthnot (1729), Whytt (1758), Heberden (1767), Pringle (1775), and many others.

Except for one republication of a Latin text, all the remaining titles are translations: 13 from German, 5 from French, 3 from Greek, 2 from Latin, 1 from Arabic, and 1 from diverse tongues. Pride of place must be allotted to the three titles from the Greek. In 1844–47 the Society published Francis Adams's three volume translation of the seven books of Paulus Aegineta, in 1849 his two volume translation of the genuine works of Hippocrates, and in 1856 his translation of the extant works of Aretaeus, including a recension of the Greek text. A distinguished translator from the Greek and the recipient of honorary degrees from Aberdeen, Glasgow, and Oxford, Adams chose to remain a country doctor at Banchory despite the offer of the chair of Greek at Aberdeen. His translations of Paulus and Aretaeus are considered definitive; no one has essayed them since, and it is doubtful whether a physician of our own century could do so. Although translations of Hippocrates have continued to appear, and room remains for debate about which of his works are "genuine," Adams's version has not been superseded and is still the most accurate and readable one. The value of the Sydenham Society's publication of these translations is enhanced by Adams's scholarly introductions in which he explains the guiding principles of Greek medicine and by his critical annotations which resolve many textual problems. For these books the scholarly world owes a great debt to the Society.

The translations from the Latin are in a class with those from the Greek despite the fact that they are of the works of English rather than classical authors. William Greenhill's scrupulously edited text of Sydenham's *Opera Omnia* was published in 1844, one of the Society's first issues. R. G. Latham's translation of this text, published in 1848–50 in two volumes, is complete and authoritative. William Harvey's *Opera Omnia* had been edited by Mark Akenside for the Royal College of Physicians in 1766, and although there were only minor problems about the Latin text, earlier translations of *De motu cordis* and *De generatione animalibus* had failed to do them justice. Robert Willis's translation for the Society was both accurate and literate, and the volume, published in 1847, also contains both disquisitions addressed to Jean Riolan and nine letters from Harvey to important correspondents as well as the first publication of Harvey's will. Harvey, a pre-Commonwealth scientist, wrote fluently in Latin, but Sydenham, a Restoration physician, wrote in English, then had his writings translated into Latin.

Another of the Society's publications from the ancient literature was Greenhill's translation of Rhazes' *De peste* as *A Treatise on the Small-pox and Measles* (1848). John Channing's translation of the Arabic text into Latin had been published in 1766; Greenhill's English version is lucid and, although a second order translation, is made valuable by his annotations and bilingual glossary. Also to be numbered among translations from ancient tongues is the compilation by John Erichsen of *Observations on Aneurism, &c.* (1844), excerpts on the subject from the writings of Galen, Aëtius of Amida, Fernelius, Gorraeus, Paré, Gullemeau, Lancisi, Alexander Monro, Haller, Heister, William Hunter, and many others. It is a convenient source book for observations up to the end of the eighteenth century.

Almost half the titles published by the Society were translations from the German. Notable among them is Justus Hecker's *The Epidemics of the Mid-*

dle Ages (1844), Babington's translation of the Berlin medical historian's essays on the black death, the dancing mania, and the sweating sickness, originally published in 1832–34. Theodore Schwann's *Microsocopical Researches into the Accordance in Structure and Growth of Animals and Plants* coupled with Matthias Schleiden's short essay, *Contributions to Phytogenesis* (1847), both seminal texts on cell theory originally published in 1839, were an important prelude to Virchow's cellular pathology, and the four volume edition of Karl von Rokitansky's *A Manual of Pathological Anatomy* (1849–54) from its recently published German text (1842–46) was another milestone in pre-Virchowian basic science. At the same time the Society published von Kölliker's *Manual of Human Histology* in two volumes (1853–54), and a few years earlier it had included on its list Hasse's *An Anatomical Description of the Diseases of the Organs of Circulation and Respiration* (1846) and Simon's *Animal Chemistry &c.* (1845–46), both of them important recent texts.

Recognition of the newly developing discipline of neuropsychiatry can be found in the publication of Ernst von Feuchtersleben's *The Principles of Medical Psychology* (1847) based on his lectures of 1845 and Moritz Romberg's *A Manual of the Nervous Diseases of Man* (1853) in two volumes, a fundamental text in neurology, the first edition having appeared in 1840, the second in 1851. Information on parasitology was supplied by Küchenmeister's two volume text *On Animal and Vegetable Parasites of the Human Body* (1857) translated from the second German edition of 1855. Incorporated in the second volume was von Siebold's short treatise *On Tape and Cystic Worms,* &c. translated by Thomas H. Huxley.

Completing the list of the Society's translations from recent German texts were Wedl's *Rudiments of Pathological Histology* (1855), a single volume with fewer details than Rokitansky's manual, Oesterlen's *Medical Logic,* a book dealing systematically with theories of epistemology and formal logic applied to medical topics, and a single volume containing both Unzer's *The Principles of Physiology* (1771) and Prochaska's *Dissertation on the Functions of the Nervous System* (1784), the latter a forerunner to the Bell-Magendie controversy, both republished by the Society in 1851 because of their historical importance.

Of the five books translated from the French, two were by Dupuytren who had been surgeon-in-chief at the Hôtel Dieu in Paris from 1815 until his death in 1835. From his *Lêçons Orales de Clinique Chirurgicale* (1832–34) the Society published *On the Injuries and Diseases of Bones* (1847) and *On Lesions of the Vascular System, Diseases of the Rectum, and other Surgical Complaints* (1854). A somewhat more recent surgical text was Velpeau's *Treatise on the Diseases of the Breast and Mammary Region* (1856) which had appeared in Paris in 1854. A few years earlier the Society had published *Observations on Surgical Diseases of the Head and Neck* (1848), a selection of articles from the *Memoires* of the French Royal Academy of Surgery. Probably the most important French text chosen by the Society for translation was P. C. A. Louis's *Researches on Phthisis, &c.* (1844); this fundamental set of personal observations on tuberculosis first appeared in 1825, and a translation into English had been published at Boston in 1836. The Society's version was from the second French edition (1843).

The reputation of its officers and the high quality of its first year's offerings (Hecker on Epidemics, Louis on Phthisis, and Sydenham's Works in Latin) launched the Society well. The initial press run of 1750 copies fell a bit short of demand, and it proved necessary to have a second printing of 500 copies. The press run for 1846's offerings was 2250 copies; using the annual reports to calculate subscriptions, back orders included, at least 2160 of them were sold. The list of subscribers in 1848 has over 2000 names including 124 foreign subscribers, 95 of the latter in the United States. The Society's books were being widely distributed and found their way into the hands of leading members of the medical profession in the English-speaking world.

But signs of decline can be detected from the annual reports. There were 2105 immediate subscribers for the 1845–46 titles, but the number fell slowly from year to year: 1821 for 1847–48, 1502 for 1848–49, 1424 for 1849–50, 1360 for 1850–51, 1270 for 1851–52, 1138 for 1852–53, 986 for 1853–54, 821 for 1855–56, and finally 673 for 1856–57. Subscriptions for back issues kept total sales above 1500 copies up to 1851, but by 1856 only 1027 copies of the year's issue were sold. The relentless downhill slope of the sales chart does not appear to have modified selection of titles, methods of promotion, or means of distribution.

The annual report for the year ending 1850, that eighth year which proved to mark the Society's half life, reviewed operations:

> The number of members ... is the measure of the extent to which this diffusion [of medical literature] has been effected ... [T]he Society's publications have been distributed among 2543 members of the profession ... Those who have been members of the Society from the beginning, have now received, for their seven annual subscriptions of £1 1*s*., twenty-three 8vo volumes, which have been got up in excellent style; the paper, printing, and binding being the best that could be obtained without entailing any extravagant expenditure. Each volume has on an average 475 pages; the whole comprising 11,000 very full pages of letterpress, and twenty-six illustrative plates. This ... is as much, if not more matter than similar societies, with a far greater number of members, have been able to issue. The ordinary publishing price of the Sydenham Society's works would probably be about £16 or £17 ...

The tone is a bit self-congratulatory, even self-justifying, but the facts as stated are accurate. However, the stated facts tell only part of the story. The figure of 2543 subscribers represents all doctors who had ever subscribed, even those who dropped their subscriptions after a year or two. The same report lists only 1424 subscribers for the current year's issue. Even today one senses that the report tried to put as good a face on the state of affairs as possible. But the books were truly a bargain—for the man who wanted them. The officers and council may have been a bit apprehensive about declining membership, but the report is whistling in the dark, albeit still in a major key.

The Society's annual balance sheets are rather less informative than the subscription lists. Although not planning a commercial venture, beyond question the Society's organizers projected that sales would cover expenses and leave a small cushion, money which might be used to subvent a publication with limited sales, e.g. a bibliography of British medical literature. The annual reports mention such a project, but the idea had to be abandoned. However, the profit and loss statements do outline the rise and fall of the Society's fortunes. A profit of £125 at the end of the first year's operations (1844) rose to £570 two years later. The high water mark was reached in 1851 when the treasurer reported a favorable balance of £750, but as subscribers decreased, the balance fell below £500 in 1854, then below £250 in 1856. But at no time was the Society insolvent; it met all its obligations, and when the remaining stock was auctioned in 1858, the books showed a modest profit.

The above records demonstrate that the Sydenham Society began under favorable auspices, was reasonably successful for a few years, then fell out of favor with its readership, and finally discontinued its operations. Certainly to attract subscriptions from over 10 per cent of the men practising medicine in Great Britain was a promising start. When we ask why the Society failed to retain its readers, the obvious point of inquiry is the nature of the books it published: What was wrong with its list? To distinguish between "scholarly" and "practical" books is invidious, but one can divide the Sydenham Society's publications into two groups—books of a classical nature or of historical interest *versus* books on basic medical science or directed at clinical problems. As a general rule, the latter appeal to a wider audience than the former, and the use of sales of popular books to finance publications of interest to the few is as old as the book trade. The Society's publications are evenly divided between the two groups. Without doing semantic violence, the texts of Hippocrates, Aretaeus, Paulus Aegineta, Rhazes, Harvey, Sydenham (both Latin and English) can be called "classics," and the essays by Hewson, Hecker, Unzer, Prochaska, the collections dealing with aneurysm, influenza, and puerperal sepsis, plus Hunter's engravings can be considered works of historical interest, a total of 15 titles.

The other fifteen titles can be subdivided into seven texts on basic medical science (Feuchtersleben, von Kölliker, Oesterlen, Rokitansky, Schwann and Schleiden, von Siebold, Wedl) and eight which deal chiefly with the diagnosis and treatment of clinical disease (Hasse, Küchenmeister, Romberg, and all five titles from the recent French literature). It is quite pleasant to sit in a library a century and a quarter later and point with pride to the enduring value of classics and the advantage of an historical perspective, but a subscriber in the 1840's and 1850's anxious to read texts which would equip him to solve problems encountered in daily practice could not indulge such a luxury. Medical libraries were few and far between, and medical journals were far from numerous. Most Sydenham Society subscribers were practitioners, but only one-fourth of the Society's books served their most pressing needs. One may regret that doctors busily engaged in caring for the sick do not have much time to peruse classical texts, but this has always been the case. Medicine is, to be sure, a learned profession and a merciful calling, but few physicians in any age pretend to scholarship. The distinguished men who passed on the books for the Sydenham Society's list were gentlemen and scholars, but they were oblivious to the lesson of the marketplace.

A case in point is the Society's production of Sydenham's *Opera Omnia* in Latin, one of its first offerings, an act of piety honouring the famous clinician whose name the Society bore with justifiable pride. Latin and Greek were still the core of the curriculum in grammar and public schools, and all holders of a university degree surely had a "reading knowledge" of at least Latin. But a considerable proportion, probably a majority, of the men practising medicine and surgery in Great Britain had been trained either by apprenticeship or as apothecaries and had only a nodding acquaintance with a few words in the classical tongues. Nor is it to be believed that even of those who received a classical education in their youth a large proportion retained fluency. One may well ask how many British doctors in the 1850's would trouble to read Sydenham in Latin when the Society sent its members a fine translation in English. Nor is it Philistine to question how many dipped into Sydenham at all. One senses a deliberate archaism among the men charged with selecting titles for the Society's list, amounting almost to a declaration that although recent advances were all very well and good, classical texts and historical analyses were just as useful. At least half the titles looked backward rather than looked forward, and this did not match the utilitarianism and belief in progress which characterized Victorian science and education.

The catalogue of books sold at auction in June 1858 when the Sydenham Society wound up its affairs furnishes a partial insight. The Latin edition of Sydenham's *Opera Omnia* was published in 1844, the first year of operations. The initial press run of 1750 had to be supplemented by an extra 500 because of the enthusiasm of the original subscribers and the hopes they held for the Society. Ergo, the Latin text was sold out. The two-volume English translation appeared in 1848–50, when the Society was still able to claim about 1400 subscribers. Available records do not inform us whether the Society reduced its press run as the number of subscribers fell, and there is no information about the number of copies printed in later years. But the 1858 auction offered 103 copies of the English version in cloth and 329 in quires, a total of 432 unsold copies. It seems only reasonable to infer that the real market for Sydenham in English was a bit fewer than 1000 copies and that, had the Latin edition been published after the text was available in translation, the market for that text would have been noticeably smaller. Likewise, only a handful of the three volumes of Paulus Aegineta remained unsold, but that too was an early issue. The unsold copies of Rhazes (1848), Hippocrates (1849), and Aretaeus (1856) numbered 528, 472, and 410 respectively. None the less, it is hazardous to predict how well a given book will sell. Judgment *a posteriori* suggests that the English version of Rokitansky's masterful pathology textbook ought to have supplied a great deficiency and that Romberg's influential text on neurology would likewise have served an immediate, practical need. Both Rokitansky and Romberg enjoyed international prestige, and there were no comparable texts in English on their subjects. Romberg's text had been successful on the Continent, the first edition of 1840 having been replaced by a second edition in 1851. But 400 copies of each remained on the Society's shelves unsold until the auction. Decision, one fears, is perilous.

As the Society's membership declined, rumblings and grumblings began to appear in the medical press. The *Lancet,* never shy about controversy, opened its columns to the dissatisfied. In a pseudonymous letter dated January, 1853 complaining of poor attendance at the annual meeting and suggesting that due notice of such meetings was not given, Antepithesis asked, "Is the Council a close corporation, and like all such bodies, afraid of a little wholesome discussion? Or have a few of the Council a *peculiar* interest in keeping things snug?" (3). The next issue contained a letter signed Jos. Rogers, voicing the following complaint (4):

> I have heard the remark made that the Society's works are not sufficiently practical; and, as a general rule, I must consider the statement to be correct. But, Sir, can this be wondered at? Who constitute the Council? Learned men, I admit; witness their translations. But . . . what sympathy can, or does exist, between them and the great body of the members?—to wit, the general practitioners? Can they, do they, know our intellectual wants? How much time . . . has the general practitioner at his disposal to read abstruse publications? But it will be said, this Society is a scientific one. Granted; but we live in an eminently practical age; and with all due deference to the members of the Council . . . etc., etc.

The issue was fairly joined. Dr. Rogers of Dean Street wanted practical books. The Council of the Society had a more academic orientation. Possibly the Council felt that practical texts were the province of regular commercial medical publishers. The original prospectus hinted it was interested in publishing books "which would not otherwise be published, from the slender chance of their meeting a remunerative sale."

The *Lancet* returned to the lists the following year, and editorial comment takes pains to rebut any suggestion that its attitude toward the Sydenham Society was actuated by personal pique because James Risdon Bennett, the Society's secretary, had recently moved a resolution hostile to the *Lancet* at a recent meeting of the Provincial Association (5). Some degree of animus must have persisted, for, when the Society discontinued its work, the *Lancet* described it as "a suicidal act, the natural climax of a fourteen years' career of exclusive misgovernment," (6) and spoke of the Society's administration as a "clique," a term of anathema to that self-consciously egalitarian journal. A month later, its leading article (7) spoke of mismanagement and extravagance, claimed that the Society's work had no real value, and took a dim view of the fact that Bennett was paid 150 guineas a year and that clerks and editors were paid "large sums" out of the funds of the Society. Now that the rancor of the 1850's has evaporated, the charge that the Society's publications were impractical seems spurious and contrived, and one may well ask whether the *Lancet's* own clerks, editors, and managers worked without salary. But journalistic animus has few limits; even two years after the Sydenham Society had expired, the *Lancet,* while commenting favorably on the New Sydenham Society could not refrain from alluding to "jobbery" (8). A more temperate editor might have based his campaign on the selection of books offered to the medical profession and argued for works which would have appealed to a more general readership, but one senses that Thomas Wakley and his staff had decided to nail down the lid on the Sydenham Society's coffin. By contrast, the British Medical Journal permitted the Society a graceful exit: "We are glad to find that the extinction of the Sydenham Society has been only apparent. A new Society is in the process of organisation; and . . . its first public meeting will be held on Monday evening next" (9).

Who killed Cock Robin? Probably a multiplicity of factors in concert and in sequence. First, a policy of book selection pitched at a level above the intellectual needs of a wide readership. Second, "personality conflict"; the Society's secretary managed to antagonize the editor of the most vocal and most anti-Establishment medical weekly. Third, the natural impatience of the Young Turks with the Old Guard. Given the atmosphere of change which culminated in the Medical Act of 1858, the Sydenham Society was a predestined victim. Quite accurately, the *Lancet* had noted that its officers and council were self-elected and self-perpetuating. Their policy could not move with the times, and the Society died of a circulatory disorder.

The New Sydenham Society was founded in 1858. In his memoir (10) of the New Society, Sir Jonathan Hutchinson recalls the concluding meeting of the original Society with Sir John Forbes in the Chair. When the motion was proposed that the Sydenham Society had completed its work, Sir Jonathan demurred that although the Society had possibly completed its work with respect to classical medical literature, there was still a wide and useful field for it in respect to translations from modern continental works. Sir John pointedly remarked that "if some young men thought the Society's work was not finished, they had better form a new one for themselves." The Old Guard occasionally surrenders. At the instance of Dr. Sedgwick Saunders, Sir Jonathan met with him and Dr. Bevill Peacock, and the New Sydenham Society was founded in short order. Among the slate of officers and members of the council appear the names of Sir Henry Acland, Thomas Addison, John Erichsen, Sir Henry Holland, Thomas Watson, and others who were at one time or another identified with the original Sydenham Society (11). Conspicuously absent are the names of Benjamin Guy Babington and James Risdon Bennett. Jonathan Hutchinson was the New Society's first secretary and remained in that post for the rest of the Victorian era.

It is not surprising that the New Sydenham Society pursued many of the best ideas of its predecessors, especially providing translations of new medical texts from the Continent. Under its aegis British medical readers were introduced to the work of Billroth in surgery, Casper in forensic medicine, Charcot in diseases of the central nervous system, Cohnheim in pathology, Donders in refraction. Frerichs in liver disease, Griesinger in psychiatry, Laveran in malaria, Hebra in skin diseases, Trousseau in clinical medicine, and many other important European writers. Classical texts and books of historical interest were replaced by such popular items as the *Biennial Retrospect of Medicine, Surgery and*

Their Allied Sciences, the *Atlas of Illustrations of Pathology*, and the *Selected Monograph* series. These find their contemporary counterpart in such serials as *Yearbooks*, and *Recent Advances*. Best of all, when the Sydenham Society closed its books, there was a balance of £300 which it donated to the New Sydenham Society (12).

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Chemotherapy of Neoplastic Diseases in Germ-Free Mice*

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This report has been prepared as an indication of respect, admiration, and affection for Dr. William A. Antopol. The individual members of this Laboratory were the beneficiaries of his advice and service for over a decade. If any indication of progress should come from our efforts, he made a significant contribution to it.

We wish to describe some chemotherapeutic trials with congenitally-infected germ-free mice which develop spontaneous, virus-induced neoplastic diseases. They are the AKR strain (1) which develops lymphatic leukemia and the Haas strain (2) which develops an immunoproliferative syndrome. The AKR mouse strain was obtained from Dr. John Trentin, Baylor College of Medicine, Houston, Texas. The Haas mouse strain was obtained from Dr. Wallace P. Rowe, National Institutes of Health, Bethesda, Maryland.

The two strains of germ-free mice were derived from conventional mothers by caesarian surgical procedures which have been described (3). They were foster nursed on germ-free C3H mothers until weaning age, and then they were propagated by normal procedures through 20+ generations, under germ-free conditions. This means that they were free of detectable macroparasites, protozoa, bacteria, and mycoplasma. Leukemogenic virus was found in all of 15 mouse strains (germ-free and conventional) which had been subjected to examinations by electron microscopy and by radiation-induced leukemogenesis (4). In addition, three strains of germ-free mice have developed breast carinomas spontaneously which contained "B" type particles, suggestive of mammary tumor virus (5). A third virus, lymphocytic choriomeningitis (LCM), was demonstrated only in Haas strain mice (6). All of the mice were susceptible to the oncogenic effects of methylcholanthrene, and the resulting fibrosarcoma cells carried "C" type virus particles as passengers (7, 8).

The germ-free mice were housed in sterile plastic Trexler-type isolator systems (9), in which they were provided sterile air, food, water, bedding, and other equipment used for the experimental procedures. They were thus maintained "germ-free" for the duration of each experiment; and the axenic status was reaffirmed by test prior to the completion of each experiment. We use the term "germ-free" with the understanding that the mice are free of "horizontally transmitted" microbial agents and that they are persistent carriers of the "vertically transmitted" viruses noted above.

The drug used in these trials was cyclophosphamide, CP (Cytoxan®), which was acquired from the Mead Johnson Co., Evansville, Indiana. It was provided as sterile dry powder, 500 mg per ampule, which was repackaged aseptically in

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smaller quantities in sealed glass ampules. Sterile drug and saline diluent, in sealed glass ampules, were transferred into the germ-free isolator by spraying the ampules with 2% peracetic acid while in the double-entry lock. The CP was dissolved in saline just prior to inoculation and administered to the mice intraperitoneally, according to their weights.

Initial trials with graded doses of CP revealed that germfree mice and rats could tolerate larger subtoxic doses of the drug than conventional counterpart animals (10), because the latter suffered and died of bacterial septicemia, pneumonia, and enteritis. Germ-free mice and rats were spared that complication because they were free of responsible microflora; however, the CP-treated mice developed alopecia and depletion of the lymphoreticular tissues.

Leukemia in Germ-Free AKR Mice

Germ-free and conventional AKR mice showed the same manifestations of leukemia as regards age-incidence, symptoms, and lesions. Over 95% developed the disease at average age of 8 months (range 3 to 12 months). Symptoms appeared in the form of rough fur, kyphosis, and dyspnea. They rarely lived beyond ten days from the onset of clinical disease. At autopsy examination, the thymus was very much enlarged, and engorged with anaplastic lymphoid cells. There was no uniformity of lesions in other organs; but in order of declining frequency, infiltrating lesions were found in spleen, lymph nodes, liver, kidneys, and lungs. Leukemia occurred in about half of the sick mice.

Two trials were instituted in AKR mice: (a) treatment before onset of symptoms, and (b) treatment after onset of symptoms.

(a) Forty-five germ-free AKR mice at age five months were administered via I.P. route 100 mg CP/kg body weight at weekly intervals over a period of 8 months (11). All of the mice survived up to the thirteenth month when the treatment regimen was discontinued. At 1 and 2 weeks thereafter, the mice were killed for examinations. Gross and microscopic evidence of leukemia was observed in seven of them within 2½ months after cessation of treatments. Of 30 conventional control AKR mice, all died within 12 weeks after onset of treatments: ten of them were leukemic and all of them showed evidence of pneumonia and enteritis.

(b) As soon as a germ-free AKR mouse showed symptoms of leukemia, it was inoculated I.P. with CP at the following schedule: 250 mg/kg body weight immediately, and 100 mg/kg body weight at weekly intervals thereafter (10, 11). The 40 treated mice survived far beyond the expected time of death; 20 (50%) of them died within 66 days (range 20 to 160 days) after onset of symptoms; and 14 (35%) survived without evidence of leukemia from 40 to 150 days after onset of treatment when the experiment was terminated. Six of the mice died in the course of the weekly injections, and all showed cell depletion of the lymphoreticular tissues. Saline-treated sick AKR mice died within seven days after appearance of leukemic symptoms; and all showed gross and microscopic lesions of leukemia.

Immunoproliferative Disease in Germfree Haas Mice

All progeny of this strain manifested life-long LCM viremia (10^3 I.C. LD_{50}/ml blood). They showed no clinical, gross, or histological evidence of disease up to age 5 months. Thereafter, the lymph nodes and spleens were swollen and contained expanding germinal zones with numerous globulin-staining cells (6, 12). The Peyer's patches were enlarged, but they contained very few germinal zones. The cortical areas of the thymus were depleted of thymocytes. Perivascular aggregations of lymphoid cells were observed in all of the visceral organs, which became more prominent with time. Many of the infiltrating cells were plasma cells, with prominent Russell bodies which stained for gamma globulin by immunofluorescence. Serum globulin levels were elevated to levels suggestive of a monoclonal gammopathy. The glomerular capillaries were relatively cell-free, became amorphous and ground-glass in appearance, and eventually fibrotic. Examinations by electron microscopy revealed that the glomerular capillaries were occluded by globulin-staining, homogeneous, fibrinoid material (13). Arteritis has been noted in some of the tissues (14). Few of the affected mice survived beyond age 12 months. At autopsy the tissues revealed extensive tumors which were classified as myelomas or reticulum cell sarcomas, which were in addition to the lesions noted above.

Three protocols of therapy were instituted with the germfree Haas strain mice: (a) groups of mice at 3 months of age were administered via I.P. route 100 mg CP/kg body weight at weekly intervals for 9 months; (b) groups of mice at 5 months of age were administered the same weekly dosage schedule of CP for 7 months; and (c) 7 mice at 11 to 13 months of age were administered 100 mg CP/kg for body weight for 3 weeks. Control Haas strain mice were administered sterile saline. Thus, three stages of the disease process were treated: before development of lesions, after onset of lesions, and at an advanced stage of disease. The results of the above noted experiments can be expressed, as follows: In Group (a), 35 mice were killed after 20 to 36 weeks of treatment. All were viremic, but none showed evidence of disease; and their serum globulins were within normal limits. In Group (b), 24 mice were killed after 20 weeks of treatment. None of the CP-treated mice showed tissue evidence of disease and serum globulins were reduced; but all mice were viremic. Benign adenomas were observed in some of the lungs. Typical LCM associated lesions were observed in the saline-treated control mice. Although few mice in Group (c) were available at this age level because of the life-limiting LCM disease, after three weeks of treatments the tissues showed some minimal evidence of disease, serum globulin levels were elevated, and the mice remained viremic. There was no evidence of drug toxicity in the mice, except for alopecia and involution of the lymphoreticular tissues; and all CP-treated mice survived for the duration of the trials (15, 16).

In an additional trial the weekly treatment schedule was stopped, and groups of the mice were examined at intervals during the next several months. All mice which were examined periodically after an interval of four months from the last inoculation of CP showed increasing evidence of immunoproliferative disease along with the persistent viremia.

A group of Haas strain mice was treated intermittently with CP for five successive days at intervals of three months. They were killed and examined three months after the third series of treatments. They showed new and extensive lesions of reticulum cell sarcomas without the visceral lesions usually associated with LCM infection in this mouse strain (17). It appears that a new lesion developed which was not sensitive to CP, and which was as serious as the disease to which the CP was directed. A group of germ-free LCM-free Swiss-Webster mice was subjected to the same protocol of intermittent CP therapy, and they developed only lung adenomas, some with histological characteristics suggestive of carcinoma.

The aim of the above noted protocol was to determine if animals with autochthonous proliferative lesions would be "cured" by the administration of maximum subtoxic doses of cyclophosphamide. Probably the results will concur with therapeutic trials involving other known immunosuppressive, cytotoxic drugs of similar class. Leukemia in conventional AKR mice has been subjected to extensive chemotherapeutic trials by Skipper and associates (18) whose results have shown that cures were elusive. The relapse rates were high which was related to the dynamic nature of the disease in mice (19). This contrasts sharply with some successful therapeutic trials in mice with transplanted tumors, in which the host animal serves only as a nonparticipating carrier of the tumor cells. Thus far, the prophylactic and therapeutic trials on germfree AKR leukemia have failed to produce cures, even with maximum subtoxic doses of cyclophosphamide, because the basic disease process was untouched.

Administrations of CP prevented the appearance of LCM lesions, and did reverse those lesions that had already developed. Here again the LCM pathogenic processes were dynamic; and when derepressed by cessation of treatments, they reappeared in the affected animals four months later. This coincided with the original incubation period for appearance of lesions. In both the AKR and the LCM syndromes the treatments were directed at the lesions, not at the etiologic agents, as was clearly exemplified by the persistent LCM viremia throughout the therapeutic trials. This was contrary to the reported enhancement effect of CP on Sendai virus infection in mice (20).

A desirable goal in therapeutic trials is maximum drug effect on the disease with minimum damage to the host. A greater benefit, perhaps a cure, would come from treatments directed at the basic mechanism through which the proliferative lesions evolved. As yet, these are not understood. Germ-free animals with naturally occurring tumors provide the investigator with ideal controlled test systems for determining therapeutic benefits, without the infectious complications from the environment. Under such conditions we can determine more definitively if a drug has merit. If a drug should show curative effects on primary malignant lesions, even in mice, the results would be very rewarding (21).

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The Identity Crisis of Pathology

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Dr. William Antopol personified a generation of successful pathologists in America at a time when this field was in its prime. A student of Dr. Paul Klemperer, Dr. Antopol preached the gospel in the style of his master, whether it was in diagnostic pathology or in research and whether to gain recognition for his field or himself. These were happy years for pathologists, and only in the last years questions arose in this nation and in the entire world as to the identity of the field. At a time when the Flexnerian principle of the crucial importance of biologic science in medicine and medical education was challenged, when sociologic and psychologic considerations came to the foreground of our thinking, when environmental pathology appeared more important than molecular pathology, William Antopol was personally able to ride the wave; but he was aware of the change in the prestige of pathology to which he was dedicated. It is, therefore, an appropriate tribute to him to review the present identity crisis of pathology. At the same time, it may be appropriate to review the role of Mount Sinai Hospital in this development—the hospital where Dr. Antopol received his original training—and Mount Sinai School of Medicine where he served with distinction as a Professor while being Director of Laboratories at Beth Israel Hospital.

Pathology, through the years, has been of major importance in the pursuit of scientific medicine and had crucial significance particularly in the last century when biology became the accepted cornerstone of medicine, so well expressed in the Flexner principle. Pathology guided medical education and practice for a long time, but shortly after this principle was formulated and announced in the early part of this century, our discipline began to give way in importance to the other basic sciences, especially as a result of the phenomenal advances in physiology, biochemistry and, recently, molecular biology. Thus, the prestige of pathology and its attraction for bright young men lessened.

During the 1960s the Flexner principle was challenged and social science assumed great importance as a basis of medicine, resulting from the demands of society in the health crisis. Even the prominence of molecular biology was challenged by environmental biology as a result of the stress on our environment. And so pathology appeared to be less and less relevant, to use a modern cliché, as the scientific basis of medicine. Today, our discipline suffers from an identity crisis in America, in Western Europe, and even in the Eastern countries.

Pathology is conventionally designated as the science dealing with the mechanism of disease, disease of the cell, of the organ, of the organism, and of population groups. Historically, however, the emphasis was on morphologic ap-

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pearance, or the shape, with the functional element being a supporting rather than a primary aspect.

At the dawn of biologic sciences, the first observations were directed toward the shape and thus morphology was born. It took a long time before recognizable aberrations of the shape were associated with an unfavorable state of the individual, with disease. The first recorded human dissections were the attempts of the Egyptians to ascertain the weight of the heart, and only later, about 200 B.C. in the late New Kingdom, did they become interested in the different appearance of other organs. Subsequently, most interests were devoted to pathology and dissection of animals.

The Babylonians, and after them the Etruscans, dissected sacrificial animals in order that their soothsayers, called haruspices, could predict the future from the appearance of organs and, particularly, from the lobulation of the liver. Thus, vases of antiquity show warriors listening to predictions by the haruspex about the outcome of the war before going to battle. While the ancient Hebrews rejected human dissection, they were among the first to apply comparative pathology, and they eliminated animal carcasses from human consumption if there was evidence of disease.

The Greeks established the relation between science and disease, expanding previous Chinese and Egyptian observations not necessarily known to them. Hippocrates became the father of Western medicine by concentrating on clinical observations in about 400 B.C. A branch of his famous, more than 3000-year-old tree on the Mediterranean island of Kos will adorn the lobby of the Annenberg Building of the Mount Sinai Medical Center. We hope that from this small stick will rise a big tree as a symbol of the connection between the foundation of medicine by Greek scientists and philosophers and the Mount Sinai School of Medicine.

Only during the Hellenic period in Alexandria around 250 B.C. was anatomy established as a science under Herophilus and Erasistratus when even human vivisection was permitted. The first recorder of medical data was the Roman Celsus at the time of Christ, and he was the first master of what today we call special pathology. He was followed by Galen, who, through his interest in functional aspects, pioneered clinical pathological correlation, although biologic science was still interwoven with philosophy.

The causes of disease and the relationship between the alterations in shape, the morphologic changes, and the functional manifestations of the disease remained haphazard, indeed speculative, during medieval times. Hypotheses and preconceived ideas prevailed over observation in the Western world. Arabian scientists carried the banner of medicine, but their drawings showed more speculation than truth. They claimed, for instance, that the portal vein brings air to the liver and that the spleen secretes bile into the stomach.

The Renaissance brought back observation as a prime tool of biology. Benivieni is credited with the first comprehensive treatise, in 1507, dealing with the unusual and miraculous causes of diseases and their cures. This was indeed the precursor of the classical work of Giovanni Morgagni published in 1761 *De* *sedibus et causis morborum per anatomen indagatis* (The seats and causes of disease investigated by anatomy). In 1960, when the New York Academy of Medicine published a facsimile production of Benjamin Alexander's English translation of 1769, our own Paul Klemperer provided a preface, an introduction, and a new translation of five of Morgagni's letters (1). This work made pathologic anatomy the basis of clinical medicine and the dissections in the famous amphitheatre in Bologna made this city the Mecca of medicine of the Western world. Autopsies there were conducted under the motto *hic locus est ubi mors gaudet succurrere vitae* (here is the place where death serves to aid the living). Under the same words we did the autopsies in the old amphitheatre of The Mount Sinai Hospital in the Lewisohn Building, and the inscription was transferred to the present temporary amphitheatre as the keynote under which our Department of Pathology has worked. Hopefully, the same leitmotif will adorn the new pathological amphitheatre in the Annenberg Building, for this motto expresses the correlation between anatomical observations and clinical management, a correlation which fluorished in the classical years of medicine in Leiden, where Boerhaave introduced bedside teaching; in London, where this correlation was exemplified by the work of Bright and Addison in the 18th and 19th century; in Paris, by way of the studies of Laennec; and in the golden age of pathology in the German and Austrian universities; while in this country it was subsequently symbolized in the clinical pathological conferences introduced by Cabot and brought to perfection in this institution by the cooperation of George Baehr and Paul Klemperer.

In the last century, in the golden age of pathology, Virchow in Berlin established as the key to disease the alteration of cells as units of life, while the master prosector, Rokitansky, in Vienna, emphasized the alteration of tissue fluids, and both pointed to the usefulness of chemical studies (2). In the meantime, diagnostic study of material obtained at operation or by biopsy was being examined under the microscope, and pathology was thus applied to the management of the living patient. The brilliant Viennese surgeon, Billroth, is considered the father of surgical pathology.

As biochemical and bacteriologic investigations gradually assumed equal value with morphologic study in diagnosis and management of the patient, the pathologic laboratory served as a useful location for the fledging clinical biochemistry, bacteriology, and, soon, hematology. While the nonmorphologic laboratory procedures assumed increasing significance in this century, the explosive development of biochemical and biophysical techniques created complex but highly effective diagnostic methods and a wonder world of machines tied to computers which would have appeared utopian only a few years ago. These techniques not only assisted in the diagnosis of individual patients but also provided information as to the basis of disease, and, therefore, were soon shared by clinical departments with pathology. In fact, it is now often argued where this laboratory medicine belongs organizationally.

Simultaneously, morphologic observations by conventional techniques were becoming less likely to lead to new discoveries since most of the morphologic observations possible in man had already been made over the years. This led to a split of pathology into the old-fashioned anatomic pathology, primarily morphologic, and the new clinical pathology, primarily technical and instrument-based, and few were the men who could master both clinical pathological correlation based on anatomic observations and the ever more sophisticated apparatus which became the backbone of clinical pathology and embraced microbiology, biochemistry, serology, hematology, and biophysics. No wonder the clinical disciplines, like internal medicine or pediatrics or surgery, and basic sciences, like biochemistry and microbiology, made claims to these glamorous and effective specialized clinical laboratories. By contrast, in most institutions, particularly in this country, anatomical pathology (in England called morbid pathology) became less attractive except as a tool to decide whether a cancerous organ should be removed.

This pulling of pathology into different directions was compounded in recent decades when complex tools of morphologic observations were developed, such as the electron microscope. These tools permitted the pathologist to investigate subcellular structures, particularly in correlation with chemical analysis of isolated fractions of the cell obtained by ultra-centrifugation. The organelles were thus defined as specialized subunits of the cell capable of carying out defined physiologic functions. This led to morphologic recognition of dysfunction of living cells and created organelle pathology, a term formulated in this institution (3). It defines the target of investigative pathology of today as being between the cellular pathology of Virchow and the still embryonal molecular pathology of Pauling, a term he introduced when he described the changed amino acid sequences in sickle cell anemia.

Shape, which had lost its fascination in biology, was reintroduced in what we once called the renaissance of morphology (4), and investigative pathology thus became again a hard basic science. It became apparent that cells are not bags of fluid, but are subdivided into compartments. Thus, processes at interphases, at the cellular, organelle or intra-organelle membrane were favored targets of basic research. Biochemical analysis done without concern for localization and interphase, was compared with analysis of the rubble of a demolished building to obtain information about its former rooms (5).

The unbelievable progress in immunology in the last decade has been largely accomplished by pathologists with ultrastructural techniques and concern with reactions on interphases and membranes. However, while clinical pathology demanded expertise in technology and thus pulled in one direction, now the introduction of basic science principles exerted a new diversion of morphologic or anatomic pathology. Up to then morphologic pathology was an empiric and conceptual science based on morphologic or anatomic observations at different levels and by various, sometimes quite complex, techniques. The thoroughness of the observation was the measure of success. The search was made for correlation on the basis of association, but to express it simply, if a story at the autopsy table or at a clinical pathology conference sounded good, it was considered to be true. The computer may eventually place observational pathology on a sounder basis and require less of the observer's memory, but it will still only demonstrate an association. Moreover, the observational pathologist who does and should concentrate on the diagnostic problem of a patient may be overstimulated by every new incidental observation at the autopsy table or the microscope and this may divert him from his planned research project.

Pathology as basic science may use the same morphologic techniques, supplemented by chemical and physical ones, but, by contrast, investigative pathology requires inductive thinking, absolute scientific proof rather than association and correlation, and with it a design of experiments in which one of two possible answers is correct. The pathologist as clinical scientist makes diagnoses and correlations, and as basic scientist looks for absolute proof and should not be diverted by incidental observations from his chosen experimental design. Investigative or academic pathology looks for understanding of diseases and rationale of therapy, in short, for scientific breakthoughs, but it contributes little to the individual patient and to the education of the students to treat the patient. Pathology is, therefore, pulled in three directions. In many institutions it is divided in two or even three different departments, sometimes geographically separated. This fragmentation of pathology, the science dealing with the mechanism of disease, prevents the necessary feedback of its different branches —diagnostic pathology, investigative pathology, and clinical pathology or laboratory medicine. It interferes with the training of pathologists to do service and, particularly, to teach the discipline to medical students and, in postgraduate training, to physicians.

With the development of biopsy techniques for various parenchymal organs, which permit us to ascertain their structure and function during life, clinicians have taken over the pathology of their field in many institutions. This is the identity crisis of pathology all over the entire world. Dire predictions are being made about the future of our discipline and they have discouraged young graduates from entering a field, the purpose of which confuses them.

In closing, let us look at the record of pathology at Mount Sinai and our attempt to solve the identity crisis. In the years before and between the world wars, when Mandelbaum, Moschowitz, and George Baehr worked in our Department of Pathology and Libman was associated with it, clinical pathological correlation based on meticulous pathologic observations resulted in the golden age of Mount Sinai Hospital where as many disease entities were recognized as in almost all the rest of the United States.

When Paul Klemperer took over the Department of Pathology at Mount Sinai in 1926, so ably assisted by Dr. Otani, the splendid tradition of correlative pathology culminated in the description of many more disease entities. But already by that time the hospital had decided, with Klemperer's wise concurrence, on the creation of independent laboratories in the areas of clinical pathology, and this prophetic decision resulted in the first clinical-chemical, hematologic, and biophysical laboratory in any hospital in the United States.

Mount Sinai has been amply rewarded by the great research achievements of these independent laboratory departments, successes of which we all are proud, and I only cite the Shwartzmann phenomenon, still a persisting influence on medical thinking.

Paul Klemperer himself, however, enlarged correlation by conceptual thinking based on thorough morphologic examination. He recognized the diseases of the until then neglected intercellular connective tissue system (6). By deliberate extension of the empiric to the conceptual approach, he created the concept of collagen disease, which has dominated and enriched pathology, clinical medicine, and also basic science ever since. His success as a meticulous empiric and incisive conceptual pathologist has made our Chair of Pathology world-famous.

At the time of Klemperer's retirement, the identity crisis, the dilemma between empiric and inductive pathology, raised its head and the challenge demanded decisions. Since it appeared impossible for one man to do justice to the whole field of empiric pathology and to carry out inductive research as a basic scientist, specialization in one organ or one area was favored after initial meticulous training in the entire field of anatomic pathology.

With emphasis on research and diagnostic service in one specialized area, it appeared possible to master empiric observations in the entire field of pathology, with inductive pathologic investigations in one area based on the knowledge of all applicable facts and techniques in basic science and the clinical aspects of this field. This approach makes a man or woman a specialized diagnostician and sought-out consultant who gives clinical service in his area of competence. This is particularly important when interpretation of biopsies requires knowledge of all aspects of the diseases of the organ in question. He thus is concerned with the problem of the individual patient and is, therefore, recognized by students and house staff as a practicing physician. With the creation of our medical school, this image has become important to give the young generation an example. As the pathologist carries out routine services in his area, he is at the same time doing research in this field. As an inductive pathologist in a circumscribed field, he should produce research in basic science hopefully matching in clarity of experimental design and incisiveness of results those of other basic scientists with whom he should be able to talk as an equal. As a recognized master of some diseases, he should remain relevant in any turn which medicine and health science may take.

The history of pathology at Beth Israel Hospital is the history of William Antopol and his coworkers. It illustrates the achievements of one man who knew how to adapt his group's approaches to the demands of the times. Meticulous morphologic studies eventually became combined and replaced by biologic endeavors, sometimes away from the mainstream of medicine, by pharmacological investigation, and, recently, by concern with environmental problems—particularly the ravages of illicit drug use. He always maintained, however, the viewpoint of the pathologist, particularly in his teachings.

The identity crisis in pathology sketched above is thus illustrated also by the lifework of this man who showed the way to meet it. Like any crisis, it is a challenge; it is a challenge for the younger generation who learn by the way it might be resolved.

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Hormones and Resistance with New Observation on the Detoxication of Ketamine

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It has long been recognized that steroid hormones participate in the regulation of growth, reproduction and general metabolism; yet, it is only in the course of the last three decades that we have become aware of their participation in diverse nonspecific adaptive reactions, particularly in the maintenance of the body's resistance to stress.

Although it is fairly easy to understand that these substances can produce specific biologic responses, such as an increase in the BMR, vasoconstriction or lactation, it is much harder to explain through what mechanism they can carry the largely nonspecific message of simply "improving resistance" against virtually any disturbance in organs throughout the body, which is essentially what "stress hormones" appear to do.

There are a limited number of ways that the body can deal with a toxic substance. It can adapt to a poison, although the latter remains unchanged in the organism, or it can get rid of it through destruction and/or excretion.

We have been interested for a long time in helping the body in this fight through treatment with "stress hormones" or "adaptive hormones," that is, substances the organism normally produces in an effort to treat itself during emergencies.

In this connection, it has been found convenient to classify the adaptive steroids into two main groups which control essentially dissimilar defensive processes:

1. *Syntoxic steroids* (from "syn" = together) are essentially tissue tranquilizers in that they create a state of passive tolerance which permits a kind of symbiosis with the pathogen.
2. *Catatoxic steroids* (from "kata" = against) actively attack the pathogen, usually by accelerating its metabolic degradation.

The systemic action of the syntoxic steroids is mainly of the "life-maintaining corticoid" type. These hormones are particularly efficient in restoring the nonspecific stress resistance of adrenal-deficient organisms to normal, but then a plateau is reached above which tolerance cannot be raised easily. Only in some instances (e.g., damage due to endotoxins, inflammatory irritants, immune reactions) do syntoxic glucocorticoids increase tolerance far above normal because, here, "disease" is primarily due to active morbid reactions of the tissues, not to direct tissue damage by the exogenous aggressor.

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Catatoxic steroids act primarily by inducing aggressive reactions which inactivate toxic substances (e.g., by accelerating their metabolic degradation). They not only restore deficient resistance (as do glucocorticoids after adrenalectomy) but are capable of raising it far above the norm. Indeed, the most active members of the catatoxic steroid group can completely inhibit the toxic effects of digitoxin, indomethacin, phenindione, nicotine, various pesticides (such as dioxathion and EPN), barbiturates, steroid anesthetics, meprobamate, picrotoxin, certain carcinogens, and many other drugs (1).

The broad spectrum of protective effects against so many agents, widely differing in their chemical structure and the type of damage they produce, clearly shows that the actions of catatoxic steroids—like those of the syntoxic steroids—are not specific.

Curiously, syntoxic effects are virtually limited to glucocorticoids, whereas the catatoxic properties appear to be quite independent of any other known steroid hormone action.

There are many overlaps, however, between syntoxic and catatoxic steroid actions. Thus, stimulation of inflammation may lead to topical degradation of the irritant by enzyme activation in the inflammatory focus; furthermore, under certain circumstances, the primarily syntoxic glucocorticoids may also enhance the hepatic detoxication of barbiturates. Yet, the distinction between these two categories appears to be as justified as, say, the distinction between glucocorticoids and mineralocorticoids because, despite some overlaps, usually, individual steroids act predominantly by eliciting one or the other reaction form.

Furthermore, available evidence suggests that the two types of defense are mediated through essentially distinct biochemical mechanisms. For example, homeostatic reactions to endotoxins or inflammatory irritants appear to depend upon the stabilization of membranes which isolate toxic enzymes that are preformed within the cell. Thereby, they protect against damage resulting from a kind of autointoxication by natural substances liberated under the influence of an aggressor. Conversely, many catatoxic hormones have been shown to induce "multi-purpose" (that is, relatively nonspecific) microsomal liver enzymes which destroy endogenous or exogenous toxic substances. Yet, without additional evidence, it would be hazardous to ascribe all catatoxic actions to the same mechanism. Not all catatoxic steroids induce enzymes against the same set of substrates; for example, certain steroids inactivate digitoxin but not indomethacin or vice versa, others inactivate both these drugs. There is evidence also that some catatoxic steroids induce drug-metabolizing enzymes in extrahepatic tissue. Finally, spironolactone offers an extraordinary degree of protection against systemic intoxication with corrosive sublimate ($HgCl_2$), presumably because the sulfur-containing group of this steroid forms nontoxic complexes with mercury.

These findings suggest interesting clinical applications in the prevention of diseases caused by exogenous or endogenous intoxications.

Based on the observations just mentioned, we undertook to examine the influence of various steroids upon ketamine intoxication in comparison with

TABLE I

Various Factors Influencing Ketamine Anesthesia

Treatment[1]	Sleeping Time[2] (min.)	Mortality[2] (Dead/Total)
None	89 ± 3	1/20
PCN	52 ± 4***	0/15 NS
CS-1	68 ± 8*	2/15 NS
Ethylestrenol	24 ± 1***	0/15 NS
Spironolactone	79 ± 8 NS	4/15 NS
Norbolethone	55 ± 6***	1/15 NS
Oxandrolone	72 ± 5*	0/10 NS
Prednisolone-Ac	49 ± 5***	1/15 NS
Triamcinolone (1 mg)	101 ± 7 NS	5/15*
Progesterone	119 ± 6***	4/15 NS
Estradiol	77 ± 7 NS	1/15 NS
Desoxycorticosterone-Ac	110 ± 6**	6/15*
Hydroxydione	96 ± 6 NS	3/15 NS
Thyroxine	107 ± 7*	10/20***
Phenobarbital	38 ± 5***	1/15 NS
Diphenylhydantoin	54 ± 8***	2/15 NS

[1] The rats of all groups received ketamine HCl (13 mg/100 gm body weight in 1 ml water, ip, once on the 4th day).

[2] Sleeping time was determined immediately after injection of the toxicant (Student's t-test), and mortality was listed on the 5th day of the experiment ("Exact Probability Test").

NS = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.005$.

Underlined asterisks indicate aggravation of toxicity.

several control substances, such as thyroxine (known to affect numerous forms of microsomal enzyme induction) as well as phenobarbital and diphenylhydantoin (two of the most potent nonsteroidal enzyme inducers of this type).

Ketamine is a general anesthetic which has been recommended for operations on certain types of patients (2). It is structurally related to phencyclidine. Although the latter substance may cause convulsions in monkeys at high doses, no such effect has been observed following treatment with ketamine (3).

Materials and Methods

Female Sprague-Dawley rats (Canadian Breeding Farms & Laboratories Ltd., St. Constant, Que.), with an initial body weight of 100 gm (range 90–110 gm), were maintained exclusively on Purina Laboratory Chow and tap water, divided into 16 groups (of 10–20 animals each), and treated as outlined in Table I.

The following steroids were tested for possible protective or sensitizing effects:

PCN [3β-Hydroxy-20-oxo-5-pregnene-16α-carbonitrile (Upjohn)],

CS-1* [9α-Fluoro-11β,17-dihydroxy-3-oxo-4-androstene-17α propionic acid

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* For Catatoxic Steroid Number 1. (Manufacturer's code number: SC-11927.) The first nonhormonal steroid shown to possess catatoxic activity.

potassium salt (Searle)],
Ethylesterenol (Organon),
Spironolactone (Searle),
Norbolethone (Wyeth),
Oxandrolone (Searle),
Prednisolone acetate (Roussel),
Triamcinolone (Lederle),
Progesterone (Schering),
Estradiol (Roussel),
Desoxycorticosterone acetate (Schering),
Hydroxydione sodium hemisuccinate (Pfizer).

All the steroids were administered at the dosage level of 10 mg in 1 ml water (homogenized with a trace of Tween 80), twice daily po., throughout the period of observation. Only triamcinolone was given at the individual dosage level of 1 mg, because of its high toxicity.

L-thyroxine (B.D.H.) was injected subcutaneously (in the form of its sodium salt) at the dosage of 200 μg in 0.2 ml water, once daily, and phenobarbital sodium (B.D.H.) and diphenylhydantoin (Eastman) at the dosage of 6 mg in 1 ml water, twice daily po., on the same days as the steroids.

Ketamine·HCl (Parke-Davis) was administered at the dosage of 13 mg/100 gm body weight in 1 ml water, ip., once on the 4th day of treatment with the previously mentioned substances.

The term "sleeping-time" is used here to describe that period after administration of ketamine during which the animals are deprived of their righting reflex. It was estimated immediately after injection and the statistical significance of the results determined by comparison with the control animals according to Student's "t" test. Mortality was listed on the 5th day; the rates were then arranged in a 2 × 2 contingency table, and the statistical significance of the apparent differences between the control and pretreated groups computed by the "Exact Probability Test" of Fisher and Yates (4, 5).

Results

As indicated in Table I, ketamine sleeping-time was considerably influenced by several of the hormonal and nonhormonal substances tested.

The most active catatoxic steroids of this group, particularly PCN, ethylestrenol and norbolethone, offered highly significant protection, whereas the shortening of the sleeping-time obtained with CS-1 and oxandrolone was barely significant. Spironolactone also reduced the length of anesthesia but not significantly. Curiously, prednisolone—which, in previous tests, proved to be a predominantly syntoxic steroid with comparatively weak catatoxic properties (1)—gave highly significant protection against ketamine whereas triamcinolone, a pure glucocorticoid, did not.

It must be kept in mind, however, that (as previously stated), because of its toxicity, triamcinolone could be administered only at the individual dosage level of 1 mg, whereas all the other steroids were given at 10 times this level.

It is not unexpected that both progesterone and desoxycorticosterone acetate, which have inherent steroid anesthetic properties themselves, prolonged ketamine sleeping-time. Yet, inexplicably, hydroxydione—which is likewise a potent anesthetic steroid—did not share this action of the previously mentioned hormones.

Thyroxine, at the high dosage level given, slightly prolonged sleeping-time, perhaps mainly as a consequence of its toxic metabolic effect. Both phenobarbital and diphenylhydantoin, known to be highly potent nonsteroidal hepatic drug-metabolizing enzyme inducers, greatly diminished ketamine sleeping-time.

In view of the fact that, at the dosage level given, only one out of 20 control animals succumbed under the influence of ketamine treatment, it could not be shown whether any of our pretreatments offered protection against the lethal effect of overdosage with this anesthetic, but triamcinolone and desoxycorticosterone induced a barely, and thyroxine a highly, significant increase in mortality.

Discussion

The experiments reported in this communication offer yet another example of the considerable modification of toxicity and pharmacologic activity that can be obtained with certain steroidal and nonsteroidal substances.

As demonstrated by earlier observations (1), some other anesthetic and hypnotic compounds (e.g., cyclobarbital, hexobarbital, progesterone, cocaine, ethylmorphine, meprobamate, tribromoethanol, trichloroethanol, thiopental) are also readily detoxified by most of the active catatoxic steroids, including the predominantly glucocorticoid prednisolone, whereas triamcinolone offered protection only against the nervous effects of cyclobarbital among all the drugs just mentioned.

As previously suggested, this may have been due to the fact that triamcinolone is a virtually pure glucocorticoid, with no demonstrable catatoxic properties, whereas prednisolone, though predominantly glucocorticoid, does also exert some catatoxic effects. Another point to consider is that the absolute dose of triamcinolone was always only 1/10 of that of prednisolone. Yet, in terms of glucocorticoid potency, even the higher amount of the latter steroid did not equal triamcinolone. Hence, the lack of protection against these drugs affecting the nervous system cannot be related to insufficiency of dosage as regards glucocorticoid and syntoxic activity.

Summary

After a general outline of the protective actions of syntoxic and catatoxic steroids exerted against various toxicants, recent pertinent experiments are reported. These showed that the sleeping-time produced in rats by ketamine, given intraperitoneally, is considerably shortened by a variety of catatoxic steroids, particulary PCN, ethylestrenol and norbolethone. On the other hand, pred-

nisolone (a predominantly glucocorticoid and syntoxic substance, which also possesses some catatonic activity) likewise protects against ketamine anesthesia, whereas triamcinolone (a purely glucocorticoid and syntaxic hormone) exerts no such effect under our experimental conditions.

Steroids possessing inherent anesthetic activities themselves, such as progesterone and desoxycorticosterone, prolong ketamine anesthesia, whereas nonsteroidal compounds, known to be potent stimulators of hepatic microsomal drug-metabolism such as phenobarbital and diphenylhydantoin, offer excellent protection. Conversely, thyroxine increases the toxicity of ketamine.

These experiments furnish yet another example of the intense modifications in drug activity that can be elicited by various steroidal and nonsteroidal compounds whose catatoxic effects against many other toxicants had been amply demonstrated by earlier investigations.

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Sensory Parameters in Communication among Coral Reef Fishes

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The coral reef community offers some unusually fine opportunities for the study of interactions among fishes. The waters are normally clear and provide good visibility. Many species of fish tend to aggregate in reef areas and one can observe many facets of both intra- and interspecies encounters. Field observations can be made of a variety of behavior patterns, including territoriality, predator-prey interactions, and virtually all phases of reproductive behavior from courtship to parental care. Extensive observations can be made with the aid of Scuba equipment and underwater motion pictures, and also with such devices as underwater television, hydrophones, and other electronic equipment.

There are a number of approaches available for the study of animal behavior. Each approach can be thought of as a kind of strategy for obtaining information, and one such strategy consists of making observations of animals under natural conditions. At first such observations are purely descriptive, but questions of interpretation soon occur. Based on purely descriptive information, however, interpretations of the mechanisms and antecedents of behavior have to be derived entirely by deduction. Cross-sectional, short-term observations of animals whose history is unknown are clearly inadequate even for deductive interpretations. Assumptions are often made to compensate for these inadequacies, and sometimes the assumptions are inadvertently included as part of the descriptive terminology. The question of the "cleaner" fish comes to mind (1). In virtually all descriptions I have ever seen of this behavior, it is rarely called "feeding" or some such operational term. The observer immediately sets up the assumption of function by naming it "cleaning" behavior, demonstrating a bias on his part that being clean is good for man and fish.

Superficial similarities to behavior of other species can also lead to misinterpretation. The colors of coral reef fishes have had many functions ascribed to them, including mimicry, camouflage, territorial defense, and courtship signals. In most cases, these functions are only assumptions made by the observer on the basis of similarity to behavior in other species. For example, the human observer can distinguish among different species on the basis of color and pattern. In some cases, he can even tell the sexes apart. It seems obvious to him that if he can tell who is who, then the fish should also be able to do this. This is a logical assumption, but it is only an assumption. It should be treated as a

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hypothesis and tested. To the human observer, the information for species or sex discrimination may be present, but this does not mean, necessarily, that the same information is detected and utilized by the fish. In a surprisingly large number of cases, insufficient data are available for interpretations to be made concerning interactions among fishes.

An excellent example of drawing confusions from insufficient evidence can be found in the widely publicized book by Lorenz (2). Based on a few short observations in the Florida keys and perhaps other coral reef areas, Lorenz came up with some sweeping generalizations about the behavior of coral reef fish. He stated, for example, that he never saw any aggressive behavior between members of different species; that demoiselles (pomacentrids) only attack members of their own species; that the more aggressive fish are always more brightly colored. Aside from the anthropomorphic assumption about aggressive motivations in the behavior of fish, it is well-known to skin diving scientists that such statements are only partially correct. The attack behavior of demoiselles, especially species of *Eupomacentrus,* is directed at all intruders, even toward an approaching skin diver. The entire theory of the basis of aggression in animals was constructed by Lorenz through selected data of this sort coupled with unsubstantiated assumptions, and strong criticism of his thesis has come from many scientific quarters (3).

On one occasion, I had the opportunity to make some observations on aggressive behavior in the vicinity of some coral reefs in French Polynesia. An example of some of the data is shown in Figure 1. Characteristic features include bright colors, and lateral displays, especially the lateral movements of the pelvic areas. Such aggressive displays are usually accompanied by sounds like those that could be produced by striking hollow pieces of wood with sticks.

The cries of a new-born human infant, such as those reported by Blinick, Tavolga, and Antopol (4), arouse responses in the mother to somehow comfort the infant. The baby is assumed, by the mother, to be expressing extreme distress. Perhaps this is so, but it is also possible to interpret these birth cries as inadequately controlled respiratory movements, or as over-compensation to the new forms of stimulation in an unfamiliar world. In any case, it is important that the interpretation be made on the basis of the infant and his limited behavioral developmental stage, and not from the point of view of the observer, especially of a mother who would be emotionally involved with the situation. Certainly subsequent interactions between mother and baby will develop into various forms of communication, and crying then becomes a more clearly defined signal.

The above examples show the importance of looking at such interactions, and, indeed, all cases of animal behavior, in terms of the level of organization of the species and the behavior (5, 6). The only justification for identifying any behavior as aggressive is the superficial resemblance of that behavior to an aggressive encounter between human beings. When one fish presents a lateral display of extended fins and gaping mouth at another, this behavior clearly must

FIG. 1. Example of an "aggressive" interaction between two individuals. Photographed in the vicinity of Tahiti, French Polynesia, by W. N. Tavolga.

have ontogenetic antecedents that are quite different from those that lead one man to make faces at another. The use of the same word and explanation for these two kinds of interactions is as anthropomorphic as a Freudian psychoanalysis of a cockroach.

To go beyond the stage of description, the student of animal behavior must resort to another strategy for obtaining his information. Laboratory investigations, in which environmental conditions can be controlled, have to supplement field observations. Experiments must be designed so that quantitative data can be accumulated. Even more significantly, the history and homogeneity of the animals need to be considered and, if possible, controlled. Under replicable laboratory conditions, one can then reasonably erect hypotheses and subject them to rigorous tests that are not feasible in the field. The progress from descriptive to experimental research is a natural one, but the two strategies are not independent or exclusive, and it is necessary to remember that even the most elaborately constructed laboratory situation is not precisely equivalent to field conditions.

The stages of my own investigations of interactions among fish is a case in point. My interest began with a study of reproductive behavior in *Bathygobius*

(7, 8) and went on from there into the problem of the behavioral significance of sounds produced by fishes (9, 10). Observation alone was insufficient for such a study, and it became obvious that experiments utilizing playback of sounds would be necessary. Given the fact that individuals emit some sort of energy output into the environment, the first question is the information content of this energy. The second concerns the receiver. Is the receiver in fact capable of detecting this information, and, more significantly, does the receiver respond to the information contained in the signal? Over the past few years, my interest has centered on the detection problem. What are the capacities of the auditory system of teleost fishes?

There are several methods available for the study of sensory capacities, but all of these fall into two general groups. One is the electrophysiological technique, in which the output of the sense organ is monitored by electrodes that detect either generator potential (microphonics) at the receptor cell, or action potentials along sensory nerves and specific central nervous centers. The "wire-tapping" methods provide information on the function of the sense organ itself, or on the way sensory information is encoded in the central nervous system. If one is primarily interested in the relation of sensory capacities to behavior, however, then other kinds of approaches are more appropriate, namely, those that utilize behavioral responses.

The study of behavioral responses to signals under field conditions has many difficulties. First and foremost is the lack of control over the environment, including its confounding stimuli. In addition, unconditioned responses are usually inconsistent and not sufficiently reliable for quantitative studies of sensory capacities. To "build in," i.e., condition, some response in the field is also exceedingly difficult and undependable. To answer the kinds of questions about sensory capacities and behavior that I was concerned with, the technique of choice was to use a conditioned response in a controlled laboratory environment. Such a technique permits the application of psychophysics to the study of sensory capacities.

Psychophysics as a science was developed primarily for the study of human senses more than a hundred years ago by Gustav Fechner. In essence, it requires a simple yes or no response on the part of the subject, and the stimulus level is varied in some systematic fashion so that the threshold value can be determined. The techniques, the accuracy of stimulus measurement, and the necessary statistics have been improved greatly over the years, and the methods have been found to be applicable to nonhuman subjects as well. Thresholds for most sensory systems can now be determined with considerable precision, although it is recognized that a threshold is not an all-or-none situation. It is now accepted that a threshold is defined as that stimulus value for which there is a fifty-percent probability of a positive response (11).

The following sections represent a survey of the application of this kind of strategy to the analysis of behavior, i.e., the use of psychophysics to determine the range of stimuli to which fish can respond.

Psychophysical Studies of Audition in Fishes

Some years ago, Dr. Jerome Wodinsky and I began a study of audition in fishes (12–14). The experimental paradigm was derived from the avoidance conditioning technique developed for fish by Behrend and Bitterman (15). In brief, the animal is trained in a small tank consisting of two compartments partially separated by a barrier. The water level over the barrier is set at a height such that the fish can swim over it readily, yet will not remain there. The fish is exposed to a sound stimulus, followed within several seconds by an electric shock. The shock and sound stop only when the fish crosses the barrier, after which a period of a minute or so is allowed to elapse, and the trial repeated. After several repetitions, the fish learns to escape the shock readily, and swims across the barrier as soon as the shock comes on. After many repetitions, the fish learns to avoid the shock entirely by swimming across as soon as the sound comes on, but before the onset of the shock. Once the avoidance response becomes sufficiently reliable, the amplitude and frequency of the sound can be varied. Threshold can be determined by several patterns of stimulus changes, but the most efficient is the so-called staircase or up-down method. After each positive response, i.e., avoidance, the sound level is decreased a specific number of decibels, and after each negative response, i.e., escape, the level is increased by the same amount. This is the same technique used by the well-known audiometer of von Békésy (16), and in these studies on fish audition, a control center, the Audio-ichthyotron, was designed to regulate the trial sequence and stimulus levels automatically (17).

A sample of the results is shown in Figure 2. Threshold curves (audiograms) for four species of reef fishes indicate that there is a considerable variation in

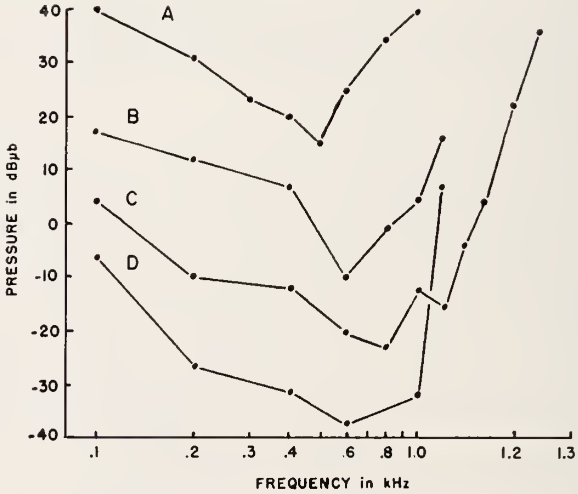

FIG. 2. Audiograms for four species of marine teleosts: A. schoolmaster (*Lutjanus apodus*, Lutjanidae); B. dusky squirrelfish (*Holocentrus vexillarius*, Holocentridae); C. longspine squirrelfish (*Holocentrus ascensionis*, Holocentridae); D. cubbyu (*Equetus acuminatus*, Sciaenidae). Each point represents a mean of several determinations of thresholds derived from data presented by Tavolga and Wodinsky (12).

auditory sensitivity. Values are given in decibels with a reference to one microbar of acoustic pressure, i.e., in dBμb. In most species tested, the greatest sensitivity is in the 400 to 600 Hz range, and above about 1500 Hz most species can be considered as virtually deaf. Differences exist even among closely related species, as, for example, a 10 dB difference between two squirrelfish, *Holocentrus ascensionis* and *H. vexillarius.* At extremes, the overall difference between the schoolmaster, *Lutjanus apodus,* and the cubbyu, *Equetus acuminatus,* is in the order of 50 dB. Comparisons among the nine species tested (12) do not seem to indicate any consistent relationship of auditory sensitivity with sound production or with swim bladder-inner ear structure.

In the case of the Ostariophysi, auditory sensitivity is clearly enhanced by the presence of the Weberian ossicles that couple the swim bladder with the inner ear. In the history of investigations of fish audition, fresh water ostariophysines have often been used as experimental animals. Quantitative data on auditory sensitivity are now available for cyprinids, or goldfish (18, 19), characids or *Astyanax,* (20, 21), and siluroids, or *Ictalurus* (22). See review by Tavolga, (23).

The problem of audition in fishes is not just a simple matter of projecting an acoustic signal into the water and obtaining the threshold by psychophysical techniques. Sound, as well as any mechanical disturbances of the medium, occurs in two forms of energy: pressure and displacement. Pressure waves can be detected as alternate compressions and rarefactions of the medium, while displacement is the actual movement or oscillation of the particles of the medium. The problem of separating these two forms of energy is particularly difficult in water, and because of the high density of water, displacement becomes an important factor. Displacement is the dominant form of acoustic energy at low frequencies and at close range to the sound source, i.e., the near-field effect, and the physics of this distinction between near- and far-field has been discussed in some detail by Harris and van Bergeijk (24) and van Bergeijk (25). In most experimental situations, where fish audition is tested, the sound source is quite close to the subject, and both displacement and pressure energy must be present.

In our initial studies on marine fish hearing (12), we found that some species consistently exhibited two sets of thresholds. Figure 3 shows a summary of the data for the blue-striped grunt (*Haemulon sciurus*) and the schoolmaster (*Lutjanus apodus*). Curves A and B were obtained in the early stages of testing, but after additional retesting of the same subjects the low frequency thresholds dropped abruptly to the values shown in curves A′ and B′. The change amounted to almost 20 dB, and it was apparent that the animals changed their sensitivity significantly as a result of the testing experience.

On the basis of these data, we theorized that this threshold shift represented a switch from one receptor system to another, and a switch from detection of pressure to detection of displacement. The swim bladder-inner ear complex is primarily a transducer for pressure waves, while the lateral line system is purely a displacement dectector. At low frequencies, the sensitivity of the lateral line is in the order of 20 or 25 angstrom units of displacement (24, 26), and calcu-

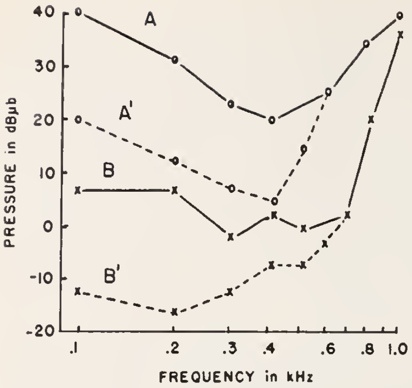

Fig. 3. Audiograms for two species of marine teleosts: A and A. Schoolmaster (*Lutjanus apodus,* Lutjanidae); B and B′. Blue-striped grunt (*Haemulon sciurus,* Pomadasyidae). Curves A and B were obtained from threshold determinations early in the testing procedure, and curves A′ and B′ represent abrupt changes in threshold obtained after several repetitions of the tests from Tavolga and Wodinsky (12).

lated displacement values for the frequencies and intensities used fell within this sensitivity range. We further concluded, therefore, that a frequencies below about 500 Hz the subjects initially utilized the swim bladder-inner ear mechanism and, after additional testing, switched to the more sensitive lateral line system. Some unexplained data still remained, however. Some individuals made this shift back and forth several times; some species never exhibited a dual threshold. Concentrating on the species that showed the dual threshold most consistently, we then proceeded to a quantitative study of the variability of thresholds in the blue-striped grunt (*Haemulon sciurus*). After over 400 threshold determinations on 23 individuals (13), the dual threshold effect could no longer be demonstrated, although the variability of the data at low frequencies was significantly high. It is possible that the bimodality may have been masked by this high variability, but in any case, we had to modify our original hypothesis. Recent experiments by Cahn, Wodinsky and Siler (27) have been able to replicate our original results and have added support to our hypothesis.

A further step in the psychophysical study of audition in fish is the investigation of the ability to discriminate sounds of different intensities and frequencies. This would be significant in relation to the function of sounds in interactions among fish. Virtually all the studies in this area have been on ostariophysines. Goldfish have been found to be capable of discriminating between two tones of the same frequency but with differences in amplitude of 3 to 6 dB (19). Pitch discrimination has been determined to be in the order of a 3% difference in frequency for *Phoxinus* (28), and goldfish (29, 30). Other fishes, i.e., those not possessing the Weberian apparatus, appear to have a much coarser discriminatory ability, although accurate data are not as yet available.

Perhaps a more significant approach to the problem of discrimination, is one in which the animal must detect a signal under conditions of considerable ambient noise. In human audition, when the noise power in a band around the signal frequency is approximately equivalent to the power of the signal, then masking takes place and the signal can no longer be detected (31). Preliminary data have indicated that this signal to noise relationship is probably similar in at least two species of marine fishes, the blue-striped grunt (*Haemulon sciurus*) and the squirrelfish (*Holocentrus ascensionis*) (32). Studies along this line are continuing, and it is hoped that such data will help elucidate the mechanism of hearing in fishes.

Another aspect to the problem of discrimination of a signal from noise is the fact that under natural conditions background noise is always present. In the threshold determinations reported thus far, laboratory conditions were controlled so that the background noise levels were at least 10 dB below the lowest thresholds at any given frequency. The noise level is measured in terms of bandwidth power, i.e., the total background noise is passed through a narrow band filter centered at the signal frequency and its levels observed on an appropriately calibrated decibel meter. Such measurements can be converted readily into spectrum level, i.e., noise per cycle, by subtracting the value 10 times the log of the bandwidth.

It would be desirable to know the extent of masking that would affect fish hearing, particularly the masking effects of ambient sea noise produced by wave motion, currents and man-made objects such as nets and boats. Information on the ability of fish to detect sounds in the presence of noise would be important in many aspects of commercial and sport fishing, especially since some experimentation is under way on the problems of attracting, repelling, and otherwise guiding fish by means of sound (33–35).

Field and Laboratory Studies on Audition in the Bone Fish

The following is a report of some preliminary studies done with a view of combining information on laboratory-determined thresholds and observations on the behavior of animals in the field. It is also an attempt to see how information of this sort could be correlated with the practical problem of fishing.

According to most sport fishermen and fishing guides, the bonefish (*Albula vulpes*) is considered unusually responsive and sensitive to sounds. This species is common in the shallow bay areas around Bimini, and fishing for them is virtually a year-round sport. Mature specimens are frequently encountered, but juveniles apparently remain in deep water. By a stroke of good fortune, a single specimen small enough to be tested in the laboratory was captured, and it survived for almost two months. It was possible, therefore, to compare the audiogram of the species with its normal sonic environment and with the sounds known to produce a fleeing response.

The test animal was a juvenile bonefish (*Albula vulpes*) 12 cm in standard length. Training was begun only after the animal had been maintained in an aquarium for about three weeks so that the probability of its survival seemed good. The subject became trained to the avoidance problem within about a week. Two other bonefish of the same size were collected at the same time, but they did not survive. The technique was essentially the one described earlier (12) in which the fish was conditioned to cross a shallow barrier in a shuttlebox in order to avoid a mild electric shock.

Although bonefish are commonly captured on hook and line in Bimini Bay, small specimens under 15 cm are rarely seen. The data given here are based on only a single subject. In spite of the limitations of the data, there is no choice, at the present time, but to assume that the results are representative of the audiory capacities of the species.

Table 1 shows the threshold determinations, using the staircase method, that were made for this single specimen of *Albula*. The limitations of the loudspeaker prevented determinations from being made below 50 Hz, and the thresholds above 700 Hz were so high as to be far above the normal sound levels encountered by the animal. At least three determinations were made at each frequency.

The data from Table I are shown in Figure 4 (curve A). A sharp rise in the audiogram is evident above 500 Hz, while the rise below 300 Hz is much more gradual. A comparison of these data with the audiograms of other marine fishes (12) shows that the fall in high frequency sensitivity occurs somewhat earlier and appears steeper than in many of the other species. The lowest threshold for the bonefish was about −26 dBμb at 300 Hz. This value is not far from the values for several marine species reported earlier.

Figure 4 also shows the spectrum of the ambient noise both in the test tank and in Bimini Bay. The plotted values are based upon measurements of narrow bands of filtered noise. Except for a 5 dB rise in the 500 to 1000 Hz range, the laboratory ambient noise was consistently about 3 dB higher than that of the quietest area of Bimini Bay. In the Bay, overall levels of ambient noise varied from −10 to +5 dBμb, depending on the specific locality. The highest levels were in the main channel, particularly during periods of rapid tidal flow. Boat noise, of course, added considerably to the background level, but all measurements were made when no boats were moving in the vicinity. Lowest background levels were obtained where the water was quite shallow, about 1 to 2 meters at high tide, the bottom was sandy with some turtle grass (*Thalassia*) but no large sponges that normally conceal numerous noise-making crustaceans. Schools of bonefish were seen often in such areas.

In areas where the bottom is littered with sponges and small coral growths, there are significantly larger numbers of small fishes and crustaceans to add to the ambient background. The chief contributors to the ambient noise here appear to be the snapping shrimp (members of the family Alpheidae). Figure 5

TABLE I

Auditory Thresholds for the Bonefish (Albula vulpes)

Frequency (Hz)	Threshold (in dB re 1 ub)
50	−17.5
100	−19.9
200	−23.7
300	−26.1
400	−24.1
500	−10.3
600	+2.0
700	+14.5

Standard deviations were all in the order of ±1.

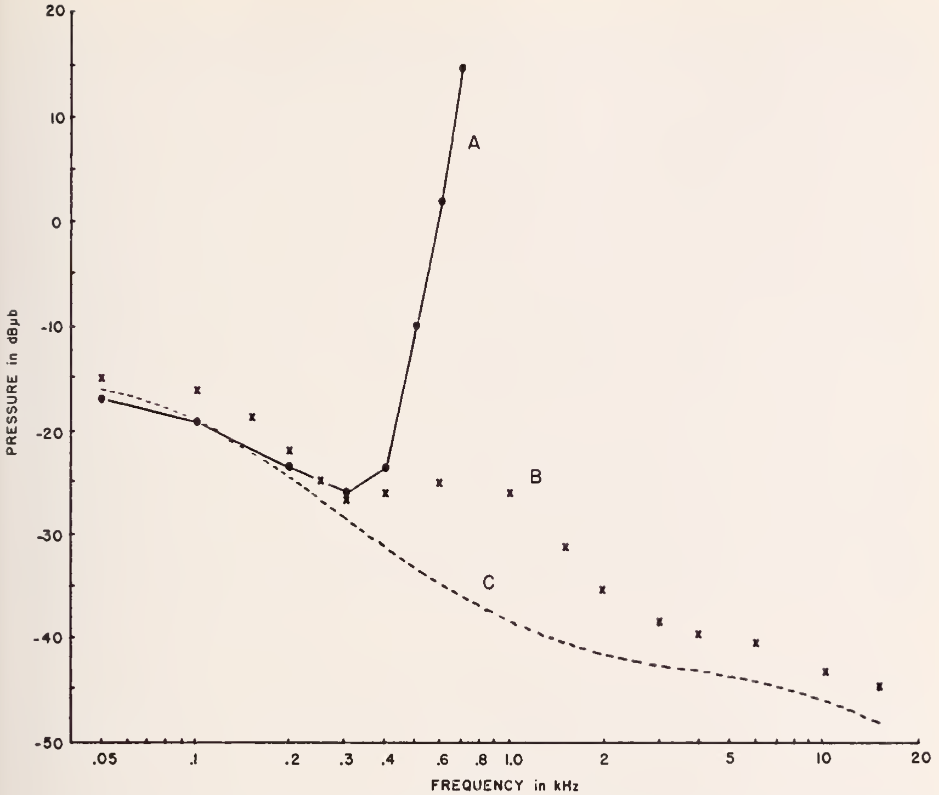

Fig. 4. Curve A represents the means of threshold determinations for the bonefish (*Albula vulpes,* Albulidae). Curves B and C were obtained from narrow bandwidth ambient noise measurements; B (×'s). spectrum of ambient noise in test tank in which auditory thresholds were obtained; C (dotted line). spectrum of ambient noise in quietest area of Bimini Bay.

(curve C) shows the added ambient noise produced by these animals, superimposed upon the quite areas spectrum. The greatest emphasis is in the range of 5000 Hz.

The spectra of various man-made noises were also analyzed. The commonest noise source was that produced by outboard motor boats, which added as much as 30 to 40 dB to the background level. Although the noise of an outboard motor effectively covered all the audio frequencies, there was always a strong dominant band that was clearly dependent upon the speed of rotation of the propeller. Thus dominant motor noise bands commonly varied from 200 to 2000 Hz.

Figure 5 also shows spectra of two common noises that might be produced during the course of fishing for bonefish. The tap of an oar or other solid object against the side of the boat (Figure 4, curve A) produces an increase of up to 20 dB of noise at a distance of 10 meters, and the spectrum of this noise shows a dominant range below 500 Hz. Curve B in Figure 5 shows the noise spectrum of a two-ounce lead sinker dropped into the water from a height of about 2 meters at a distance of 10 meters from the hydrophone. A variety of other small objects gave similar spectra, and all had a strong dominant intensity at about 300 Hz. Larger objects that splashed more showed greater intensities in the high frequencies, while smaller, lighter objects (such as a bait shrimp) gave essentially the same spectrum at a 5 to 10 dB lower intensity.

A comparison of the curves in Figures 4 and 5 shows the following: 1) that the audiogram of the bonefish below 300 Hz tracks the background noise spectrum closely; 2) that the noise spectrum in the test tank and that of the quiet area of Bimini Bay are quite similar; 3) that the ambient background of snapping shrimp and other invertebrates is probably outside the hearing range of the bonefish; 4) that many man-made noises, including slowly moving outboard motor boats, have strong dominant frequencies in the vicinity of 300 Hz.

In the range below 300 Hz, the background noise would actually mask signals of an intensity below the noise level in that frequency range. Given a lower background level, it is highly probable that the bonefish audiogram would show a significantly greater sensitivity in this low frequency range. The portion of the audiogram below 300 Hz, therefore, is to be considered a "masked" threshold, and is comparable to the effects of masking noise in studies on human

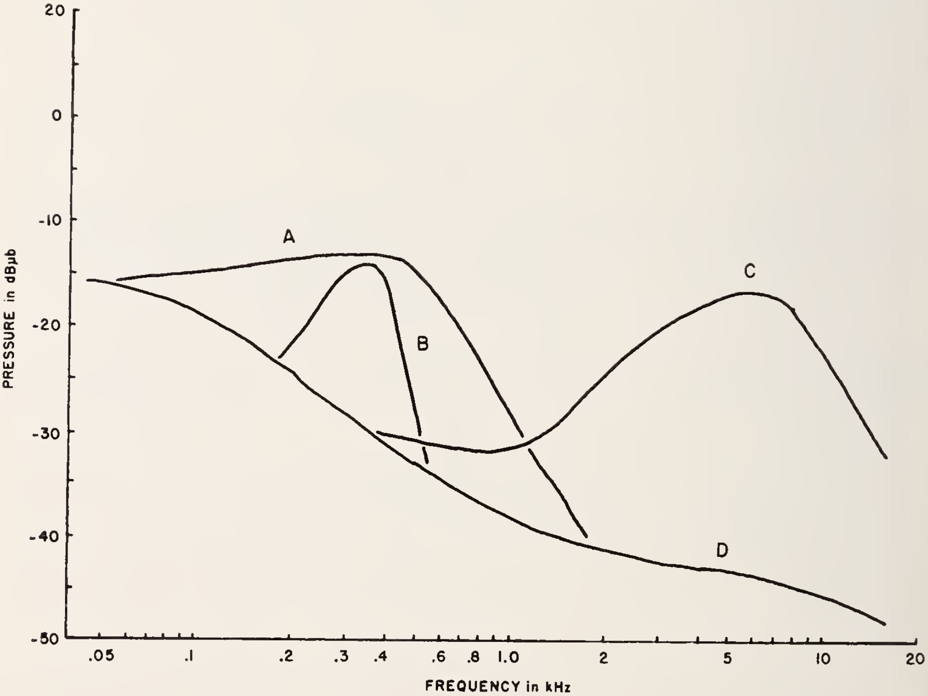

FIG. 5. Spectra, obtained through narrow bandwidth measurements, of various environmental noises: A. noise produced by a light tap of an oar against the side of a fiberglass boat (recorded at a distance of about 5 meters); B. noise of a lead sinker dropped into the water from a height of about 2 meters (recorded at a distance of about 10 meters); C. ambient noise in Bimini Bay in vicinity of sponge bed area; D. ambient noise in shallow, quietest area of Bimini Bay.

audition (36). The close relationship between the low frequency portions of ambient noise and audiogram curves is to be expected.

For practical purposes to the fish, therefore, the most sensitive range is around 300 Hz. Coincidentally, the spectra of many man-made sounds show a strong emphasis at about 300 Hz. As a result, the fish should be able to detect such noises easily.

The technique for bonefishing is unique in that the animals have to be stalked with the minimum of noise, and usually the services of an experienced guide are required, so that the fish can be seen and approached before throwing out the bait. This species has the reputation of being extremely "spooky" and they take flight at the slightest sound. My own experiences have confirmed these observations, and it is tempting to draw the popular conclusion that this species is especially sensitive to sound.

On Bimini, a successful bonefish guide is a man who not only has the ability to see the fish from some distance, but also knows, from experience, that certain noises will frighten the fish. Any thump or other blow against the side of the boat is to be avoided, and a good fisherman will throw out his bait so that it skips over the water rather than landing from a steep trajectory. The data given here support these conclusions, since these types of sounds have spectra with emphasis in the 300 Hz range.

The idea of the bonefish possessing a particularly high auditory sensitivity is not supported by the present data. Two factors combine to give this false impression. The fact that the fish are so easily frightened by many man-made sounds is the result of the coincidence of the spectra of these sounds with the unmasked portion of the fish's audiogram. Furthermore, the responsiveness of the fish is not necessarily a measure of its sensitivity to detecting the sounds that frighten it. Undoubtedly many other species in the same area are equally sensitive, but simply do not respond in such an obvious manner.

The response of bonefish to sounds of outboard motors is particularly interesting. Fishermen often relate instances where a boat moves at full speed through a school of bonefish, and the school just moves aside or splits slowly to allow the boat to go through. If, however, the fisherman slows his boat down within a short distance of the school, then the fish take fright and flee rapidly out of sight. I have observed this myself on several occasions, and noted that the greatest response of the fish took place when the speed of the motor slowed down to about 300 rpm. In spite of the high intensity of the noise of an outboard motor running at full speed, the fish do not take flight. The visual stimulus of the boat must, of course, play a role in this situation, since bonefish rarely permit an approach of a boat to a distance of less than 10 or 15 meters, no matter how quietly the boat moves.

It has also been observed that bonefish are more easily frightened in deeper water (over 3 meters) than in water of a meter or less in depth. Over the soft sand and *Thalassia* that covers much of the shallow area of Bimini Bay, attenuation of sound is greater than in deeper areas with a harder substrate.

It should be emphasized, however, that many of these conclusions are based upon the assumption that the threshold data derived from the single juvenile subject are valid. It is likely that older and larger animals would demonstrate less sensitivity, especially at the high frequency portion of the audiogram. Admittedly, the data may not be as representative of the species as would be desirable. However, since this species is so poorly amenable to laboratory investigation, the probability of repeating these determinations is quite low. These data and conclusions are presented at this time as preliminary, with the hope of stimulating further studies along the lines of relating auditory capacities of fishes to their responses to sounds in their normal environment.

Psychophysical Study of Vision in Fishes

In an aquatic environment, vision is often limited by the turbidity of the water, and it is probable that the acoustic sense is the dominant source of information from a distance. The visual system is of great significance, however, in the behavior of many species of fishes, particularly among the coral reef forms where the water is generally quite clear. The recent monograph by Breder and Rosen (37) shows the importance of visual cues in the various aspects of reproductive behavior among teleosts.

Bright colors, distinctive patterns, and characteristic movements are evidently cues in the behavior of most coral reef fishes, and often assumptions are made as to the functions of these cues on the basis of their visibility to the human observer. What are the specific visual sensitivities of fishes for color, detail, shape, movement, etc? Most of the data on these questions are derived from physiological sources, i.e., from retinal pigment absorption spectra and from electrophysiological studies. Burkhardt (38), for example, showed the relationship between spectral sensitivity, cone absorption spectra and electroretinograms in goldfish. Similar data, showing three classes of photopic units, have been obtained physiologically by Liebman and Entine (39), Witkovsky (40), and others.

Behavioral data, summarized by Walls (41), strongly indicate that most teleost fish, at least, can discriminate colors. Recent studies on the goldfish by Yager (42) have supported this through the use of conditioning techniques.

Dark adaptation increased the sensitivity by at least two log units (43), under conditions where, presumably, only the scotopic (rod) units were operating.

Dr. David W. Jacobs and I recently began a series of studies in which the avoidance conditioning technique, similar to the one used in audition, was applied to the study of vision in fish (44). In addition, the light stimulus was presented from above, simulating natural illumination, and an attempt was made to obtain actual threshold values for illumination in absolute terms. Most prior work has used only a relative scale for the determination of sensitivity, but we believed that even an approximation of light values at threshold would be useful in ecological and behavioral studies.

Preliminary data on the African mouth-breeder (*Tilapia macrocephala*) showed that after dark adaptation, the greatest sensitivity was in the green portion of the spectrum, from 500 to 550 nm (nanometers). At 525 nm, the threshold was approximated at .000023 $\mu W/cm^2$ (microwatts per square centimeter of irradiance as measured by a spectro-radiometer). This value can be converted readily into illuminance units by applying the luminosity coefficient for the CIE standard observer (45). Thus the above value becomes equivalent to about 2×10^{-5} foot-candles for scotopic vision, but it is doubtful that the standard human observer is equivalent to the standard fish observer.

It is hoped that data of this sort might help to elucidate the probable function of coloration in the behavior of fishes. Significantly, the undersea world rapidly becomes monochromatic as depth increases. The blue end of the spectrum is scattered, the red is absorbed, and the predominant spectrum even at depths of a few meters is a narrow range of green. The fact that fish can detect light of different wavelengths may be a less important question than how well can they discriminate one wavelength from another, given equal brightness.

Psychophysical techniques can also be used to answer questions about discriminatory ability, and even to test the capacity of the fish to resolve fine details or to distinguish shapes. Although some studies in these directions have been made, the field is essentially wide open, and basic information of this kind is essential to evaluate the role of vision in interactions among fishes.

Summary

In the vicinity of coral reefs, the complex community of a large variety of species of fish makes this habitat a particularly useful one for the study of behavior and, especially, interactions among individuals. The usual clarity of the water in these areas also enhances the opportunities for field observations, and this represents an important strategy for obtaining information on behavior.

Field observations alone, however, can be misleading and the interpretations of behavioral mechanisms must be done cautiously. Information contained in coloration, pattern or movement may be apparent to the observer, but not necessarily perceived or utilized by other animals in the community. The imputation of aggressive motives to fish is inappropriate and anthropomorphic. Behavior in any given species must be interpreted by the level of organization of the species, and the strategy of experimental, laboratory investigations becomes necessary.

The sensory basis for interactions can be investigated physiologically or behaviorally, and the behavioral approach is probably more appropriate in this context. Under controlled laboratory conditions, quantitative, i.e., psychophysical, measurements can be made of the sensory limits of the organism.

Psychophysical studies in fishes have demonstrated acoustical sensitivity and have provided threshold values that can be used in further behavioral investigations. Some data of this sort are now available for vision as well as audition.

The relation of signal detection to masking noise is significant because the normal acoustic environment of a fish contains a considerable level of background noise. A preliminary study is reported on the effect of sounds under natural conditions in the bonefish (*Albula vulpes*).

An audiogram, based upon a single juvenile specimen of the bonefish shows

the highest sensitivity at 300 Hz of about −26 dB (re 1 microbar). Above 400 Hz the threshold curve rises rapidly to about +14 dB at 700 Hz. Below 300 Hz, the curve rises slowly to a value of −17 dB at 50 Hz. A comparison of the audiogram with the spectrum of ambient noise in the test tank shows that the thresholds below 400 Hz follow the background noise spectrum closely. These values, therefore, probably represent masked thresholds. The background spectrum in the test tank is similar to that found in the quietest areas of the bonefish's normal habitat.

The bonefish is easily frightened by many man-made noises, and many of these, including boat-tapping, dropping of bait, and motor noise, have strong peaks in the vicinity of 300 Hz. The responsiveness of these fish, therefore, is not so much dependent upon any unusually sensitive hearing but, rather, on the coincidence of many sounds with their most sensitive range and the readiness with which the fright response can be evoked.

Acknowledgments

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Current Concepts of Acquired Human Valvular Disease

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Introduction

Acquired disease of the human heart valves has been a major problem in etiology and pathogenesis. The valves most commonly involved are the mitral and the aortic. The heart valves are essentially connective tissue structures, of unique geometry, made to withstand the hemodynamic forces associated with cardiac action. With time, heart valves may be continuously exposed to a variety of injurious stimuli, but we continue to recognize only two main categories of disease: rheumatic and arteriosclerotic processes affecting the mitral and/or the aortic valves. Valvular heart disease may be seen not only as a consequence of rheumatic carditis but also in patients with rheumatoid arthritis, systemic lupus erythematosus and other disorders frequently grouped together as the so-called collagen diseases. In fact, this association of acquired valvular heart disease in connective tissue diseases was so striking that Bagenstoss and Titus (1) discuss acquired diseases of the heart valve as rheumatic and collagen disorders of the heart.

Heart valves have a limited capacity to respond to injurious stimuli (2). Consequently, the evolving patterns of response should be reviewed in terms of better understanding the final common pathways. A variety of theories have been proposed as being most responsible for producing valvular damage. Hypersensitivity, which may be regarded as a special form of allergy, is an exaggerated physiologic response to a specific physical or chemical agent in which an initial sensitizing contact with an antigen renders the involved tissues hypersensitive to damage from repeated contact with the same antigen. Hypersensitivity phenomena, especially of the anaphylactic type, have been considered as the pathogenetic mechanisms responsible for the lesions in some of the connective tissue diseases. Autoimmunity has also been advanced as an hypothesis for the alteration of heart valves. Autoimmune valvular heart disease refers to that pathologic condition in which the pathogenesis of the alteration results from immunologic reactions between antibodies or cells produced by the individual and the tissues and cells of the heart valves in the same individual. This definition does not imply that the individual's tissues or cells are necessarily the original antigenic stimulus. That is, the implicated antibody was stimulated by an auto-antigen. The theory that the antigenic stimulus might not be

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autologous in origin implies that the antibody formed must have a capacity for cross-reaction with substances present in the individual if an autoimmune state is to result. If the antigen is autologous some mechanism must exist for the heart valve tissue to become autoantigenic. In recent years, there has been much discussion concerning the origin, mode of action and significance of antibodies of different types that are capable of reacting with host tissues or substances that have been identified in rheumatic heart disease, rheumatoid arthritis and systemic lupus erythematosus.

The direct damage of heart valve tissue by bacterial products continues to be an attractive hypothesis. Most recently, others have suggested that viruses or viral actions may also damage heart valves leading to irreversible changes. Thus, a host of toxic products produced directly or indirectly by a variety of microbial agents might also be responsible for damaging heart valves.

General Structure

Heart valves may be described as membranes covered on all free surfaces by endothelial cells. The internal composition is essentially a matrix of connective tissue ground substance in which are embedded varying amounts of collagen, elastin, nerves and cardiac muscle. A continuous sheet of endothelial cells sweeps from the atrioventricular valves to the surface of the atria and envelops the chordae tendinae as a sheath. From the chordae, the sheath of endothelial cells continues over the papillary muscles.

The controversy concerning vascular supply of the valves has not been resolved. It is clear that fetal valves have a limited vascular bed. This appears to disappear in man fairly rapidly following birth. However, in some instances, remnants of the fetal pattern may remain in the adult valve. In our experience, approximately ⅓ of normal mitral valve leaflets show a delicate vasculature in adults. The vessels do not extend beyond the proximal 25% of the valve. In effect, the leaflet is a relatively avascular structure and in its most distal aspect is not unlike the cornea.

Mitral Complex

The normal atrioventricular complex is a group of interrelated structures comprising the annulus, valve leaflets, chordae tendineae and tips of papillary muscles (2, 3). The skeleton of this complex is an uninterrupted sheet of collagen which is suspended on the annulus and anchored into the papillary muscles. Ultrastructurally this skeleton is composed of densely packed collagen fibers arranged in bundles and interspersed fibroblasts. In the leaflets the collagen bundles interdigitate in a basketweave-like arrangement as they fan out from the chordae tendineae to the annulus. In the chordae the collagen bundles which are continuous with those of the fibrosa are oriented to the long axis of the chordae. These columns of collagen penetrate the tips of the papillary muscle, branching into small and smaller units much like the roots of a tree. The individual collagen fibers eventually are anchored to the sarcolemma of the myo-

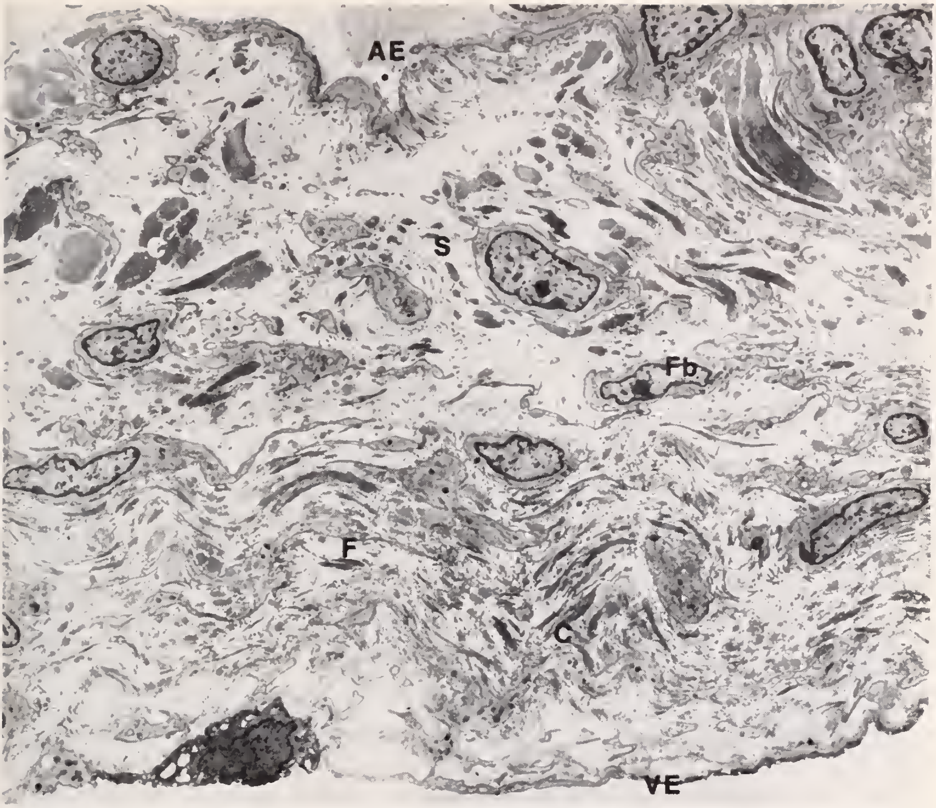

FIG. 1. The distal portion of the mitral valve leaflet is composed of a distinct spongiosa (S) and fibrosa (F). The spongiosa is an acid-mucopolysaccharide-protein matrix in which are embedded collagen bundles (C), fibroblasts (Fb), and elastic fibers. The fibrosa is the fibrous skeleton of the valve and consists of more densely packed interdigitating bundles of collagen (C) which form a discrete structure. Both the atrial (AE) and ventricular (VE) surfaces of the leaflet are covered by endocardial cells. × 5,100.

fibers of the papillary muscles by a system of microfibrils. This unique arrangement of collagen gives the complex great strength and allows the forces exerted on it during systole to be dissipated over the annulus (4).

The cardiac muscle fibers in the valve leaflets extend into the midportion. Not only are they continuous with the atrial myofibers but identical in ultrastructure. The myofibers in the valve are unique in their terminal distribution. They appear to end as rounded or blunted finger-like projections in the connective tissue matrix. The valvular myofibers are physiologically distinct and may be a site of origin of ectopic left atrial arrhythmias (5). It has also been postulated that contraction of the muscle in the leaflets is an active process serving to stiffen the closed valve during systolic contraction.

The semilunar valves also contain a collagen skeleton which extends from the annulus to the edges of the cusps. Ultrastructurally this skeleton consists of interlacing bundle of collagen fibers between which are scattered fibroblasts. The

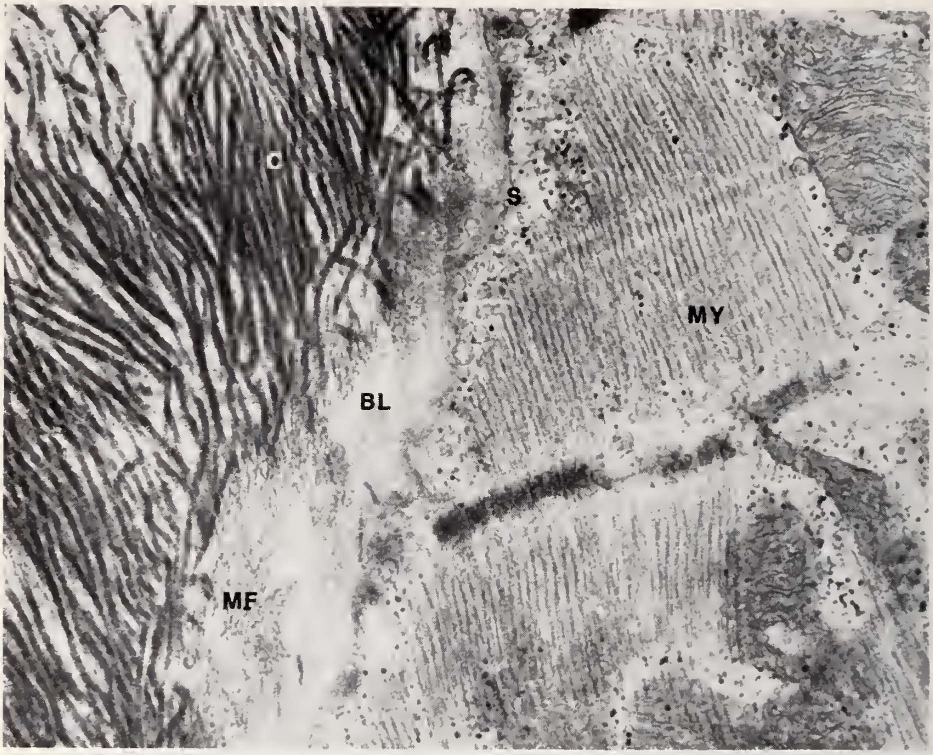

FIG. 2. In the papillary muscle, the collagen fibers (C) from the chordae tendineae appear to terminate in the basal lamina (BL) of the myofibers. Within the basal lumina numerous microfibrils (MF) enmesh the collagen fibers and appear to anchor them into the basal lamina. Myofilaments (MY) extend to the sarcolemma (S) but do not enter the basal lamina. × 39,000.

spongiosa, which is ultrastructurally similar to that in the atrioventricular valves is beneath the ventricular endocardial cells. Between the endocardial cells and the spongiosa is a specialized structure, the ventricularis. It is composed of collagen and elastic fibers and terminates at the tip of the cusp where it forms the nodule of Aranti. No cardiac muscle is present in the semilunar valves.

It is the collagen framework which serves to unite the papillary muscle, chordae tendineae, valve leaflet and annulus into an integrated, functional unit. From this it follows that damage to one or more components of the complex will result in changes in the entire complex. There are many examples of this concept in human medicine. Rupture of the papillary muscle or chordae tendineae (6) are associated with distinctive valvular changes. Fibrotic processes in the valve produce significant hemodynamic alterations relative to the annulus and chordae. From a pathological point of view, the concept of valvular disease as a secondary phenomenon, will direct closer attention to the integrity of the associated structures of the complex.

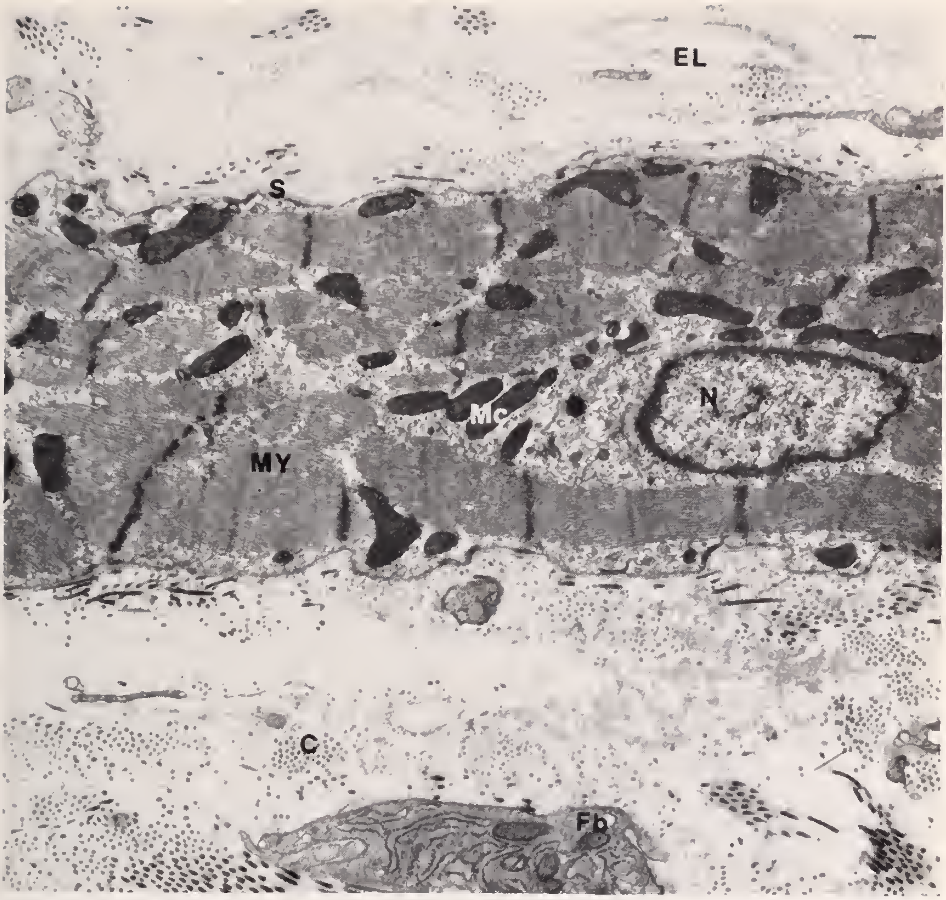

Fig. 3. In the mid-portion of the mitral valve leaflet the cardiac muscle fibers are separated from one another by an abundant loose connective tissue containing collagen fibers (C), elastic fibers (EL) and fibroblasts (Fb). The muscle cells are delineated by a distinct sarcolemma (S) and their intracellular components, myofilaments (MY), mitochondria (Mc) and nuclei (N) appear normal. × 13,500.

Connective Tissue

The connective tissue of the valve may be considered in terms of the formed elements (cells and fibers) and the extracellular matrix or ground substance. Macromolecular acid mucopolysaccharide protein complexes in the ground substance are important in maintaining the mechanical features of the leaflet and the integrity of the fibers. The acid mucopolysaccharides (AMP) in the valve function as very effective ion and water exchangers. Minor changes in the physical conformation of the molecule can result in significant shifts of water and electrolytes (7). So-called "mucoid" or "myxomatous" changes in the leaflets are expressions of increased bound water in the ground substance. Munch (8) reported that this is a degenerative process since it may proceed to progressive depolymerization, proteolysis and complete disintegration of the macromolecule.

As a consequence of increased water content in the valve the glycoprotein coat of the collagen fiber is physically altered. Previous studies have indicated that "swelling" or "edema" of the ground substance increases the susceptibility of collagen to fraying, fragmentation, and dissolution (9, 10). These connective tissue events lead to a cellular attempt to restore the valve with the deposition of new collagen. When this reparative process moves beyond normal limits, the excessive collagen stiffens the valve and sets in motion a chain of hemodynamic events.

In this regard, the possible pathogenesis of canine mitral valvular fibrosis is important (11). This is a spontaneous disease occurring in dogs mostly of the smaller breeds. The disease appears to begin as a mucoid swelling of the mitral valve leaflet with distortion of the free edge. This progresses to a slow but steady increase in valvular collagen. Mitral insufficiency develops followed by left ventricular hypertrophy and failure. At autopsy, the mitral valve is contracted, shrunken, and totally incompetent. Canine mitral valvular fibrosis is not associated with inflammation or known infectious agents. An immunological process is unlikely due to the total absence of serological or cellular immune phenomena.

Valvular fibrosis may be related to the aging process. Native collagen fibers are held in an extended, crystalline position by hydrogen bonds between the adjacent chains of a polypeptide helix with three strands. These hydrogen bonds rupture upon heating and the structure collapses into a randomly coiled form. This thermal contraction of collagen occurs because of an internal molecular rearrangement. Recent studies by Lawson et al (12) show that collagen polypeptide chains derived from young and old animals are similar as regards thermal shrinkage. However, the force of thermal contraction in the fibers of old animals is five times that of fibers from young animals. The conclusion is that old animals have an absolute increase in collagen. This may help explain the controversy concerning the studies of heart valves from older humans indicating no change with age of the collagen protein.

It is not uncommon for pathologists to observe fibrotic valves associated with a striking lack of fibroblasts or other mesenchymal cells. The assumption is that the cells have disappeared. Studies on cells which excrete protein have established that synthesis of the protein occurs in the endoplasmic reticulum and that subsequent concentration, storage, and excretion is via the Golgi apparatus. Some investigators suggest that the Golgi is responsible for collagen processing while others disagree. Trelstad (13) has studied the embryonic chick corneal epithelium by EM. He has convincingly demonstrated that the epithelium is producing collagen and excreting it into the subepithelial space. The collagen is excreted by the cell in a highly polarized manner being deposited solely beneath the basal surface of the epithelium. Trelstad (13) suggests that the corneal epithelial cells synthesize collagen in the endoplasmic reticulum, transport it to the Golgi apparatus via small vesicles, package and condense it in vacuoles and excrete it by fusion of the vacuole with the cell surface membrane.

We have alluded before to the similarity in structure between the cornea and the valve leaflet. It would be interesting to search for protein excretion systems in valvular endothelium. Perhaps the integrity of valvular collagen is maintained by an endothelial cell-ground substance interaction.

Finally, cardiac connective tissue contains a unique cell, the Anitschkow cell. Studies from this laboratory (14) have demonstrated the ultrastructure features of these cells and their potential phagocytic activity. Normal valves show rare, scattered Anitschkow cells usually near the annulus. These cardiac histiocytes are the precursors of the Aschoff cell.

Rheumatic Heart Disease—An Enigma Revisited

Progressive valvular disease as a consequence of rheumatic valvulitis continues to defy attempts at elucidation of etiology and pathogenesis. Over the past 30 years, evidence has been accumulating clearly implicating the beta-hemolytic Group A streptococci. Since these organisms were not cultured or identified in the cardiac lesions, an immunological process was advocated. During the acute phase, antibodies develop against almost all of the potent antigens in the streptococcus. Many of these cross-react with antigens of the sarcolemma or intercalated disc of cardiac myofibers. Kaplan (15) has demonstrated immunoglobulins and complement in auricular appendage biopsies or suitable postmortem material from patients with rheumatic valvular disease. Most recently, Kaplan (16) has demonstrated antibodies to valvular fibroblasts. Thus, an autoimmune mechanism seemed likely.

However, these heart-reactive antibodies from the serum of patients with rheumatic carditis have no direct toxic effect upon cultured mammalian heart cells or after *in vivo* binding to the surface antigens of the myofibers. Like other connective tissue disorders, the antibodies are epiphenomena and have no primary pathogenic role. They may reflect an immunological response to cardiac tissue damage by some unkown agent perhaps produced by the streptococcus. It is clear that infection with beta-hemolytic Group A streptococci precedes the onset of acute rheumatic carditis. This is associated with a variety of immunological responses. Exactly how these events lead to tissue damage is still unclear.

There are other alternative mechanisms. The beta-hemolytic streptococcus is an arsenal of toxic products. Streptococcal proteinase is a highly potent cardiotoxic agent. Various fractions of the bacterial cell wall are damaging to connective tissue. The ubiquitous streptococci may be responsible for repeated exposures of susceptible individuals to these direct-acting toxic materials. Subclinical cardiotoxicity is difficult to study, but the newer advances in bioinstrumentation should serve to reexplore this field by investigators.

Burch and Colcolough (17) have suggested that viruses may be the underlying cause of rheumatic carditis. This notion would fit some of the epidemiological and clinical observations. About 50% of patients with acute rheumatic carditis fail to yield positive cultures for streptococci when first seen. Only 3–5% of patients with proved streptococcal pharyngitis develop carditis. Kaplan and Frengley (18) have shown that the majority of patients with acute rheumatic fever have autoantibodies to heart muscle in their sera of non-streptococcal origin. Almost 90% of all cases of pharyngitis are viral in origin and the streptococci present may only be incidental. Upper respiratory tract infections caused by bacteria and viruses simultaneously are common. Coxsackie viruses have experimentally produced myocardial and valvular lesions (19). There are many possible streptococcal viral interactions that deserve serious investigation.

Unique to rheumatic carditis is the Aschoff body, generally regarded as pathognomonic. The Aschoff body is a cardiac granuloma which has evolved in response to focal connective tissue damage (20). In the process, Anitschkow cells are transformed into Aschoff cells. Thus far, attempts to localize streptococcal antigens and/or antibodies in the Aschoff body have been unsuccessful. Immunoglobulins appear to be present but no further analysis is available. Rheumatic valvulitis may be associated with Aschoff bodies in the mitral leaflet at the annulus, proximal, distal, and chordae insertion areas. Aschoff bodies are eventually replaced by scar tissue and these dense bands of collagen may be responsible for initiating the progressive sclerosis of the valve. Thickened blood vessels usually accompany the inflammatory reaction and persist after healing is complete.

Many questions remain concerning the etiology and pathogenesis of rheumatic mitral valvular disease. It is of interest that patients with Hurler's syndrome may have mitral stenosis (21). Grossly and microscopically these mitral stenotic lesions are strikingly similar to end stage rheumatic mitral stenosis. Is it possible that the individual susceptible to rheumatic carditis possesses some kind of hereditable connective tissue disorder?

Conclusions

An attempt has been made to present an overview of the more important current concepts of acquired valvular disease in man. Hypersensitivity, anaphylaxis and autoimmunity have all been proposed as immunological events capable of damaging valves. The mechanical and electrophysiological properties of the valve in a broader context, the valve complex, has been emphasized. Present day thinking of connective tissue reactivity in terms of end-stage valve disease stresses the integrity of valvular collagen. Finally, rheumatic valvular disease is discussed as a model for testing all of these concepts. It is a disorder unique to man, characterized by a highly specialized tissue response—the Aschoff body. Much is speculative but it is important to appreciate that many uncertainties exist in the study of valvular disease.

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Interaction between 9,10-Dimethyl-1,2-Benzanthracene and Myxovirus in the Production of Pulmonary Tumors in Mice

DANIEL LEIGH WEISS, M.D.*

Introduction

The objectives of the studies reported here are, first, an evaluation of the interaction between a known chemical carcinogen and a representative non-oncogenic myxovirus, commonly a cause of human disease, in terms of their relative activity in the production of lung tumors in intact mice. Secondly, it is hoped that these studies, in which test agents are directly applied to the respiratory tract of the test animals, would indicate an alternative bioassay system for carcinogenesis, as a corollary to the classical, long-term, skin-painting assay.

In such a complex, multivariant system as that which probably lies at the basis of oncogenesis, three separate variables have been isolated and evaluated: (1) the spontaneous development of lung tumors as an expression of the genetic load of test animals, and their age-specific susceptibility, (2) the oncogenic potential of known chemical carcinogens, and myxoviruses considered separately, and (3) the relative oncogenic potential of combinations of these agents, all systems being studied in the respiratory tract. The last study is designed to mimic the human situation of concurrent or successive exposures to chemicals and viral agents.

Materials and Methods

The test animals used throughout the investigations were mice of strain A/J and strain C57Bl/6J, obtained from the Jackson Memorial Laboratories, Bar Harbor, Maine, and maintained and bred in our laboratories under standard conditions. A group of specific-pathogen free animals of each strain was bred and maintained in our laboratories for additional studies. Samples of each group of animals were proved to be free of polyoma virus.

All test materials were instilled into the tracheobronchial tree by aspiration after light ether-alcohol anesthesia. The instilled materials were suspended in saline. Several animals in each group received the test instillate in which was suspended finely divided carbon particles to provide a visible tracer for evaluation of the extent of distribution of the instillate in the tracheobronchial tree.

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The test materials were:

(1) Influenza virus, type A, strain PR8, instilled intranasally in a standard dose of 0.05 ml. in a 1:10 dilution in saline followed in 24 hours by 0.05 ml. of saline intranasally. The raw mouse lung adapted viral hemagglutination titer was standardized at 1:640 with a MLD_{50} in the test animals of 1:40.

(2) 9,10-Dimethyl-1,2-benzanthracene (DMBA) instilled intranasally in a single dose of 0.05 ml containing 0.5 mg/ml in saline for an inoculum dose of 0.025 mg.

Results

I. Characterization of Inbred Mouse Strains A/J and C57Bl/6J Relative to the Spontaneous Appearance of Lung Tumors

Two hundred mice of each strain, equally divided by sex, were maintained under standard laboratory conditions for their normal life span. Upon death all mice were autopsied for gross evidence of the development of spontaneous lung tumors and confirmed by microscopic study of frozen sections.

The A/J strain of mouse survived an average of 17 months, with a range of 15 to 20 months, if all early deaths mainly associated with intercurrent infection are disregarded. Thirty animals died between 3 and 12 months of age of such causes. The strain appeared to be unusually susceptible to intercurrent infection when compared with similarly housed rodents of other strains. The cumulative tumor incidence in all animals was 64%. The pulmonary tumors were all adenomas.

The C57Bl/6J mice survived an average of 29 months, with a range of 24 to 32 months. Three animals died of infection before 12 months of age. The overall lung tumor incidence was 4.5%, all tumors being pulmonary adenomas.

II. Evaluation of the Oncogenic Role of Influenza Virus A (Strain PR8)

A single intranasal instillation of influenza virus, followed in 24 hours by a single intranasal instillation of saline, was performed in all test animals at 5 months of age. The inoculation was varied in one group of animals of each strain by the inoculation of intraperitoneal cortisone on the day following the viral instillation. The cortisone-treated animals showed no differences in tumor incidence from the non-cortisone-treated animals.

All mice were sacrificed at staged times over a 75-day period (3, 8, 14, 35, and 75 days) and step sections of the lungs were examined microscopically for the presence of tumors. Samples of lung were studied for recovery of the virus.

Animals which received virus contracted the infection as proved by typical histological changes and viral recovery from the pulmonary tissues within 8 days of inoculation. No viral recovery was successful after 14 days.

The lung tumor incidence of each mouse strain was calculated from examination of mice studied at 75 days only. Table I summarizes the results of the study. The mice sacrificed for study before 75 days are not included in the calculations.

The incidence of tumors in the control groups is comparable to the spontaneous tumor incidence, provided that the youth of the animals at the time of final examination is considered. The tumor incidence of the control groups represents the basal or spontaneous rates for their age.

The percentage of tumors in both strains is increased in the virus-inoculated groups, but the statistical significance of the increase is different. In the strain C57Bl/6J, the small increase in incidence is not statistically significant ($p = 0.6$). On the other hand, in the strain A/J mice, the virus-inoculated group shows a highly significant increase in lung tumors ($p < 0.01$).

A duplicate study performed with specific-pathogen-free animals of the same strains yielded comparable results (Table II).

In this case, the increased tumor incidence in A/J mice is significant at the $p < .001$ level.

These studies confirm the acceleration effect of influenza virus, type A, strain PR8, on the appearance of lung tumors, when exposure to the virus occurs at a very young age. Over the 75-day experimental period, the numbers of animals bearing lung tumors appears to parallel the development of spontaneous tumors in each inbred strain if the latter is considered over the full life-span of the animal.

III. Evaluation of Inhalation of DMBA with and without Concomitant Infection by Influenza Virus on the Respiratory Tract

These studies were performed only on A/J mice. They were selected because they were shown to have a high spontaneous lung tumor incidence and a substantial acceleration effect when exposed to influenza A (PR8) virus. Mice were treated with one intranasal instillation of either DMBA, influenza A virus,

TABLE I

Pulmonary Tumors in Mice Treated with Influenza Virus at 5 Months of Age

Strain	Group	N	N w/Tumors (75 days)	%
A/J	Control	52	9	17
A/J	Experimental	113	43	38
C57Bl/6J	Control	53	1	2
C57Bl/6J	Experimental	108	5	5

TABLE II

Pulmonary Tumors in SPF Mice Treated with Influenza Virus at 5 Months of Age

Strain	Group	Lung Tumor % (75 days)
A/J	Control	12%
A/J	Experimental	45%
C57Bl/6J	Control	4%
C57Bl/6J	Experimental	5%

DMBA plus influenza A virus, or saline alone at 8 weeks of age. They were maintained under normal laboratory conditions for 90 days, sacrificed, and subjected to complete autopsy study. All animals dying during the experimental period were similarly examined.

A variety of morphologic alterations were found which have been classified as (1) proliferative responses and (2) inflammatory and degenerative responses.

Proliferative responses: (A) Tumor. Nodular tumors were solely of the adenoma type, characteristic of the usual spontaneous lung tumor of this strain.

(B) Bronchial hyperplasia. The bronchial epithelium was either focally or generally converted from a single cell layer with underlying reserve cells to a multilayered structure, 3 to 8 cells thick. The altered epithelium varied in type from secretory columnar cells to flattened simplified epithelium to cells showing frank keratinization in areas of squamous metaplasia. Mitotic figures were found among the multilayered cells.

(C) Alveolar cell hyperplasia. This change consisted of a rounding and heaping up of the alveolar lining cells which were not extruded to lie free in the alveolar lumen. Macrophagic activity was not apparent. The cell volume was increased; the nuclei were from 1.5 to 3 times their normal diameter but maintained a normal chromatin structure.

(D) "Morulae." This term is introduced to describe proliferation of the collar of cells at the junction of the terminal respiratory pathway and the alveolar antrum, cells apparently derived from the small epithelial cells at the terminus of the bronchial system. When compared with the normal bronchiolar-alveolar junction, this collar of proliferating cells partially compromises the lumen of the orifice. The cells are small, regular epithelial elements, having no particular orientation to one another, other than being fixed at the junction. There is no evidence of tumor formation beyond the localized proliferative change.

(E) Atypia. Atypical epithelial cells were separately noted, whether associated with proliferative phenomena or not. Such cells had abnormal nuclei, were multinucleated, or showed atypical mitotic patterns. Generally, the cell cytoplasm was normal or greater than normal in volume, but the nuclear-cytoplasmic ratio did not appear to be significantly disturbed in fixed tissue preparations.

Inflammatory and degenerative responses include (A) interstitial pneumonia, acute or chronic, focal or diffuse, (B) atelectasis, either lobar or segmental, (C) focal lymphoid hyperplasia with significant lymphoblastic activity resembling the human lymphoid bronchitis of pertussis, and (D) emphysema.

Table II describes the incidence of the proliferative lesions and Table IV, the inflammatory and degenerative lesions.

Statistical analysis of the data reveals the following:

1. Combined exposure to a single dose of DMBA with influenza is a significantly better system for tumor production than exposure to influenza alone ($p < .0001$) and is somewhat more effective than exposure to DMBA alone, being at the margin of statistical significance ($p = .079$).

TABLE III

	N	N Affected	%
Tumor			
DMBA	37	3	8.1
Influenza/Saline	74	0	0
Saline	12	1	8.3
DMBA/Influenza/Saline	90	18	20.0
Bronchial Hyperplasia			
DMBA	37	5	13.5
Infl/Sal	74	20	27.0
Sal	12	1	8.3
DMBA/Infl/Sal	90	9	10.0
Alveolar Cell Hyperplasia			
DMBA	37	5	13.5
Infl/Sal	74	0	0
Sal	12	0	0
DMBA/Infl/Sal	90	10	11.1
"Morulae"			
DMBA	37	2	5.4
Infl/Sal	74	0	0
Sal	12	0	0
DMBA/Infl/Sal	90	11	12.2
Atypia (Bronchial)			
DMBA	37	1	2.7
Infl/Sal	74	6	8.1
Sal	12	10	83.3
DMBA/Infl/Sal	90	9	10.0

TABLE IV

	N	N Affected	%
Interstitial Pneumonia			
DMBA	37	3	8.1
Infl/Sal	74	27	36.4
Sal	12	12	100.0
DMBA/Infl/Sal	90	9	10.0
Atelectasis			
DMBA	37	8	21.6
Infl/Sal	74	5	6.7
Sal	12	1	8.3
DMBA/Infl/Sal	90	4	4.4
Focal Lymphoid Hyperplasia			
DMBA	37	2	5.4
Infl/Sal	74	5	6.7
Sal	12	0	0
DMBA/Infl/Sal	90	18	20.0
Emphysema			
DMBA	37	0	0
Infl/Sal	74	18	24.3
Sal	12	0	0
DMBA/Infl/Sal	90	10	11.1

2. DMBA alone appears to be a more effective agent in the rapid production of lung tumors than influenza virus alone ($p = .035$).

3. Exposure to influenza alone at 8 weeks of age produced a depression in tumor development below the rate in controls, animals exposed to saline, and animals exposed to the same virus at 5 months of age. This may be due to the extensive destructive effect of the virus on the bronchial epithelium in very young animals, removing a significant percentage of the population of cells at risk.

4. Bronchial cell hyperplasia was found in all groups of animals with no statistically significant differences of incidence between treatment groups.

5. "Morula" formation was found only in DMBA-treated animals.

6. Focal lymphoid inflammatory reaction was found in animals exposed to influenza and to DMBA. When combined, there appeared to be an additive effect.

7. Emphysema developed only in animals exposed to influenza virus, with and without the addition of DMBA.

Discussion

The phenomenon of acceleration of the development of tumors by exposure to influenza virus has been described by Kotin and Wiseley (1). The results of the studies reported here confirm these findings when the exposure occurs at a very early age.

The combination of exposure to a true chemical carcinogen and an accelerating agent, simultaneously, in a single exposure system might seem, theoretically, to be of minimal practical value. Nevertheless, this system of combined exposure produced a significantly higher tumor incidence in a short-term experiment than that produced by either the chemical carcinogen or the accelerating viral infection alone. The increases appear to be greater than a simple additive effect. The conclusion drawn from these studies is that the viral acceleration effect is imposed upon both the genetically determined spontaneous tumor rate and upon the induction effect of the carcinogen. This study also supports the observations of Imagawa et al on the acceleration effect of a combination of influenza virus and urethane (2). Martin et al (3) have also demonstrated the carcinogenic effect of several human viruses combined with carcinogens.

Accelerating agents applied in studies of potential carcinogens in the respiratory tree particularly when young animals are used as test systems, provide a more rapid means of evaluation of suspected inducers. By using very young animals (8 weeks of age at time of exposure), the accelerating effect of viral exposure on the development of spontaneous tumors in a short term study has been reduced or eliminated. This has resulted in a dramatic demonstration of acceleration effect in the combined chemical carcinogen—viral system. These observations conform with those made by many authors (4–8) regarding the advantages of studies in very young animals in the field of oncogenesis.

The mechanism of enhancement of tumor production by combined viral and carcinogen exposure is, as yet, unclear. The detrimental effect of influenza virus on bronchial mucosa has a major detrimental effect on the ciliary clearance system and may set the stage for a greater effect of an inhaled carcinogen (9).

The apparently contradictory observation of an acceleration of tumor appearance on exposure to influenza at 5 months of age and a depression on exposure at 8 weeks of age, over a short period of observation, may be related to the destructive activity of the virus upon the bronchial mucosa. The younger, smaller animals appear to be more vulnerable to the viral infection, and it is assumed that a larger percentage of the cells at risk have been destroyed. This may retard the evolution of tumors for some period of time until the entire bronchial epithelium is reconstituted and the normal cell population reaches its usual risk potential.

The morphological changes in the lungs of young A/J mice produced by DMBA include pulmonary adenomas, hyperplasia of alveolar cells, and a specific hyperplasia of the group of cells at the teminus of the bronchiolar ramifications as they abut upon the alveolar antra. The latter two changes have not been seen to progress to adenoma formation in these short-term experiments, though this possibility may be shown in more prolonged studies. On the other hand, these proliferative changes may represent evanescent phenomena which may resolve without tumor formation. Contrary to the findings reported by Kotin and Wiseley (1), no epidermoid carcinomas or adenocarcinomas were observed in either control or test animals. All tumors were typical pulmonary adenomas similar to those found spontaneously developing in untreated animals. No metastases developed during the short course of the study. The evolution of squamous metaplasia of bronchi to neoplasia was not observed, though a stable metaplastic phenomenon was seen in a significant number of animals exposed to infectious virus.

A major difference in experimental technique between these studies and those of Kotin and others was the single dose exposure used herein as opposed to multiple exposures by others. This may have introduced a variable which makes comparison of less significance.

The consistent development of lymphoreactive foci in lungs exposed to either influenza virus or DMBA is clearly an expression of the immunologic surveillance system. The persistence of these foci for as long as 90 days after exposure, long after the viral particles are recoverable, suggests that they are directed against cells which are antigenically transformed by the action of the agents employed.

The development of a hyperplastic tissue response at the junction of the terminal respiratory bronchiole and the alveolar antrum, designated as "morula" formation, on exposure to DMBA suggests that this is a particularly sensitive tissue focus. It may be comparable to other types of junctional tissue, such as the endo-ecto-cervical junction, which are particularly prone to malignant transformation. None of the animals demonstrated the evolution of a malignancy at this point, though this may have been a function of the limited time span of the study. It is not clear whether the cells at this junctional site respond in a hyperplastic manner because of peculiarity of cell metabolism or if the site tends to accumulate a greater percentage of the infused carcinogen because of the mechanics of the clearance mechanism of the mouse lung.

A striking percentage of mice exposed to influenza virus developed enough structural damage in their lungs to produce foci of emphysema within 90 days after infection. The distribution of the emphysematous areas was usually basal and anterior and in no case exceeded an estimated five percent of the total lung volume. This observation deserves further study to evaluate the possibility of progressive pulmonary destructive disease being induced by exposure to viral agents.

The system used in this study, combined exposure to a non-oncogenic virus and a carcinogen (or suspected carcinogen) in very young animals, provides another approach to the evaluation of the oncogenic potency of suspect materials. It offers the possibility of a reasonably short-term test system and uses the organ system under study in an intact animal.

Summary

The acceleration in time and incidence of the development of pulmonary tumors in mice, strains A/J and C57Bl/6J, was demonstrated by intranasal administration of influenza virus, type A, strain PR8. Strain A/J mice showed a more pronounced early development of tumors upon intranasal administration of 9,10-dimethyl-1,2-benzanthracene (DMBA) upon exposure at 8 weeks of age. Combined exposure of DMBA and influenza was more effective than either DMBA or influenza alone. A variety of other hyperplastic phenomena were demonstrated after exposure to one or other of these agents. It is suggested that the combination of exposure to a non-oncogenic viral agent and a suspected carcinogen in the intact respiratory tree of young animals is a valid system for evaluation of oncogenic potential in a short-term test period.

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Automation of Immune Hemolytic and Complement Reactions by AutoAnalyzer. I.*

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* This work was performed during 1970 and 1971 in the Automation Development Laboratory (Dr. Ferenc Hutterer, Director) of The Mount Sinai Hospital, New York.

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This is Part I of a three-part work. Parts II and III will appear in successive issues. Part II will comprise Hemolytic Titration of Complement and Its Components, Kinetic Studies of Complement Fixation, and Basic Equations for Complement Fixation; Part III will comprise Anticomplimentary Effects, Automation of Complement Fixation Reactions, and the references for the entire manuscript.

The AutoAnalyzer is manufactured by Technicon Instruments Corp., Tarrytown, N.Y. 10591.

Requests for reprints should be sent to: Walter Henley, M.D., Mount Sinai School of Medicine of The City University of New York, N.Y. 10029.

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Abbreviations and Symbols

A	Amboceptor—hemolysins, antibody against E which interacts with C
Ab	Antibody
AC	Anti-complementary substance
Ag	Antigen
C	Complement
$C1, C2, \cdots$	1st, 2nd, etc. components of complement
CH50 (CH100)	Units of complement yielding 50% (100%) hemolysis
CF	Complement fixation
CFR	Complement fixation reaction
D	Serum dilution
E	Erythrocyte = red blood cell
E^*	Erythrocyte damaged by complement
EA	Sensitized erythrocyte
GPS	Guinea Pig Serum
HD	Hemolytic Dose
MHC	Minimal hemolytic concentration
MHD	Minimal hemolytic dose
OD	Optical Density
R	Receptacle
RBC	Red blood cells
$SRBC$	Sheep erythrocytes
t	Time or duration of a reaction
T	Temperature (usually C°)
VBS 2+	Veronal buffer solution with added Ca^{++} and Mg^{++}

Abstract

This report is a summary of research and development involving complement fixation and immune hemolytic reactions evaluated by AutoAnalyzer. It includes description of adaptations by which this equipment can be used in serologic and serodiagnostic laboratories, experiments on temperature gradients, automatic serial dilutions, titration of complement components, the kinetics of complement fixation reactions, and the problem of anticomplementary sera.

Introduction

An immunologic reaction between antigens in the membrane of erythrocytes (*E*) and antibodies (*A*) specifically directed against those antigens can lead to an interaction with hemolytic complement (*C*) and, subsequently, lysis of *E*. The process of $E + A + C \rightarrow$ lysis is called immune hemolysis. It is extraordinarily complicated. Firstly, not all sources of *E* are equally sensitive to this kind of process, and, even within species, marked differences can be observed. Secondly, not all kinds of bound *A* have the property of reacting in the classic manner with *C*; IgM, a 19S immunoglobulin, is generally more efficient than IgG, a 7S immunoglobulin. Finally, *C* is not a single entity, but represents nine individual components that react sequentially in the order 1, 4, 2, 3, 5, 6, 7, 8, 9. Only after all nine have compleetd their task is lysis observed. Additionally, several components are quite unstable, leading to interruption of the lytic process. For a thorough description, the reader is referred to the text of Rapp and Borsos (1).

Despite this high degree of complexity, immune lysis is a process that can be manipulated easily to yield highly reproducible data. Some instrumentation is necessary, minimally a spectrophotometer with which to measure hemoglobin. This report will describe a more sophisticated form of instrumentation, namely, continuous flow analysis by AutoAnalyzer, whereby many aspects of immune lysis can be fully automated (2).

An AutoAnalyzer actually substitutes flow rates in plastic and glass tubing for static volumes and uses the air segmentation of a continuous flow system to maintain the integrity of the successive samples to be analyzed. This form of instrumentation is particularly useful to study immune lysis because: a) each reagent and sample is introduced reproducibly; b) incubation can be achieved under carefully controlled temperatures and for precise periods of time; c) a signal indicative of lysis or its effects can be obtained by continuous flow colorimetry (hemoglobin), turbidimetry (intact cells), or rate-metered cell counting; and d) a record of the signals reflects the data of either discrete tests or the curved results of kinetic studies.

The best known immune lytic system involves the use of sheep erythrocytes (*E*), rabbit antibodies incited by boiled sheep stroma (*A*), and fresh guinea pig serum (*GPS*) as a source of hemolytic complement (*C*). A reaction system containing *E* in the presence of an appropriate excess of *A* (*EA*) is very sensitive to minute amounts of *C*. If *C*, sufficient for *EA* lysis, is first reacted with another complement fixing antigen-antibody system, it will no longer be available for the lysis of *EA*. This is the basis for complement fixation (*CF*) tests (3).

CF usually requires an optimal excess of bound antibody. For this reason, complement fixation reactions (*CFR*) cannot be performed efficiently by the usual forms of continuous flow analysis; strong antibodies tend to contaminate successive samples. *CF* tests have, however, been automated by performing them in sampler cups which, ultimately, permit assay for residual *C* content.

1) *INSTRUMENTATION AND REAGENTS*

Technicon Sampler II. The sampler tray can be equipped with a thermostatically regulated circulating heating bath to maintain 37°C. This permits *CF* to take place in sampler cups. Other sampler trays can be iced for use when sampler cups are assayed for residual hemolytic complement. A large variety of timing cams are available. Rotary stirrers are used to mix constructed dilutions and reactions automatically.

Proportioning Pumps. Either Model I or Model II is useful. Jacketed coils are used to maintain 37°C during continuous flow mixing and incubation.

Measurement of Hemolysis. Colorimeters using 15 mm continuous flow cuvettes and red filters (630–660 nm) are useful to measure residual turbidity of sheep cells after sufficient incubation for their immune lysis. Turbidity as optical density (*OD*) is not linear, but deviates minimally from linearity between 0 and 0.3 *OD* units. Alternately, an autocounter can be used for rate metered counts of unlysed sheep red blood cells (*SRBC*). Both instruments require a removal of segmenting air bubbles from continuous flow reaction mixtures just prior to their use for evaluation. Contained volumes and flow rates needed to maintain the integrity of each test at two different rates of testing are given in Table 1-1. The calculations suppose that change in continuous flow cuvettes is exponential: $C_t = C_0 e^{-(x/V)t}$. For a 95% change ($C_t = 0.05\ C_0$), $x = 3V/t$.

Recorder. Paper speed for 120 tests/hour was 45 inches per hour instead of the standard 18 inches. *OD* ruled paper is used for turbidimetry and linear paper for particle counting.

Magnetic Stirrers are used with silicone-coated stir bars to mix sampled cell suspensions.

All reagents are standardized by automatic titration in a manner analogous to manual methods.

Diluents. Solutions are prepared in doubly distilled water. Isotonic solutions used were 0.85% NaCl, Kolmer saline and Mayer and Levine's solutions (4, 5). Veronal buffered saline (*VBS*) enriched with calcium and magnesium ions (Mayer's *VBS* 2+) was used in most experiments (6).

SRBC. In Europe commercial preparations are prewashed and concentrated,

TABLE 1-1

Minimal Contained Volumes (x) and Flow Rates Required for Colorimeter and Cell Counter

	Colorimeter		Cell Counter
	15 mm	8 mm	
Volume (V)	0.15 ml	0.10 ml	0.01 ml
Time of Analysis (t)			
15 seconds (120 tests per hour)	1.8 ml/min	1.0 ml/min	0.12 ml/min
30 seconds (60 tests per hour)	0.9 ml/min	0.5 ml/min	0.06 ml/min

and contain antibiotics. They are transported successfully by mail in plastic bags and need only be diluted in *VBS* 2+. In the United States sheep blood in modified Alserver's solution (6) is available, but these cells must be triply washed before use. Suspensions of *SRBC* are standardized by electronic particle counting or *OD* of hemolysate at 541 nm.

Hemolysin. Any commercial product active when diluted beyond 1:10,000 can be utilized. Dilutions of 1:1,000 are not made because they would be strongly hemagglutinating. In automated tests, 2 parts of complement, 1 part of *SRBC*, and 1 part of hemolysin are mixed for the hemolytic reaction.

Complement. Fresh, fresh-frozen (−20°C), or lyophilized guinea pig serum (*GPS*) is used. At a final dilution of 1:1,500, *GPS* will lyse 50% of *SRBC* in 30 min at 37°C. Undiluted *GPS* is stable, but dilutions are not.

Antigen-Antibody Reactions. Table 1-2 indicates the immunologic systems studied by AutoAnalyzer.

Inactivation of Sera. This step was not automated. Specimens were heated at 56°C for 30 min.

Quantities of Antigen and Antibody Required. In general, the amounts needed for automated tests differed little from amounts used for manual methods.

Controls. These did not differ from controls used in conventional manual methods.

2) *MEASUREMENTS IN AUTOMATED SEROLOGY*

1. *Measurement of Hemolysis*

a. *Colorimetry:* In immune hemolysis, hemoglobin liberated by lysed cells can be measured after the reaction mixture has been centrifuged and the button of red blood cells (*RBC*) discarded. Colorimetry measures the extent of hemoglobin tinting of the supernatant. Oxyhemoglobin is characterized by absorption maxima at 576, 541 and 414 nm and 541 nm is used most often. Colorimetric titration at 541 nm has only rarely been used in automatic serology (25). At the end of hemolysis, intact red cells must be agglutinated and removed. A procedure of this nature complicates experiments significantly.

b. *Turbidimetry:* Figure 2-1 indicates the validity of turbidimetric measurements at 630 nm. For final red cell concentrations less than 2 × 10^7 cells/ml, there is linearity between *RBC* number and *OD*. These mixtures were sampled and their turbidity recorded. The results are given in Figure 2-1.

c. *Cell Counting:* In the Technicon cell counter (autocounter) a red cell crosses the cuvette and is visualized because it deviates a beam of light. Lysed cells in the sample do not deviate this light beam. Finally, deviated light is focused by a system of lenses to excite a photosensitive cell so that the quantity of light measured is proportional to the number of red cells which are in the path of the light beam. A photomultiplier increases the electrical signal which is registered. The cell counter includes discrimination to eliminate background from very small particles. Thus, intact

TABLE 1-2

Immunologic Systems Studied

Antigens	Antibody	References
Parasites		
Hydatid fluid	Patient's serum	—
Amoeba histolytica	Patient's serum	7
Bacteria		
Neisseria gonococcus	Patient's serum	—
Salmonella typhi	Rabbit antiserum	8
Salmonella paratyphi A	Rabbit antiserum	9
Salmonella shottmulleri	Rabbit antiserum	9
endotoxins	Rabbit antiserum	10
Aeromonas sp.	Rabbit antiserum	—
Escherichia coli	Human antiserum	10
Brucella abortus	Bovine antiserum	11
Reiter's antigen	Syphilitic serum	12–14
Rickettsia prowazeki	Rabbit antiserum	—
Psittacosis-Ornithosis	Human antiserum	—
Virus		
Aphthous virus (animal foot & mouth disease)	Guinea pig antiserum	15, 16
Influenza	Human antiserum	17
Adenovirus 5	Human antiserum	—
Mumps	Human antiserum	—
Australia antigen	Human antiserum	18, 19
Cells		
Erythrocytes	Hemolyitc serum	9, 20
Human liver	Human antiserum	—
Thyroid	Human antiserum	21
Gastric mucosa	Human antiserum	22
Proteins		
Human gamma globulin	Rabbit antiserum	—
Egg albumin	Rabbit antiserum	—
Haptens		
Cardiolipin	Syphilitic serum	13, 23, 24

SRBC can be counted with minimal error, and the scale of measurement can be expanded to eliminate the need for preliminary adjustment of erythrocyte suspensions. Sensitivity of the cell counter permits use of 10^6 cells per ml.

The three methods, colorimetric, turbidimetric and enumeration give comparable results in serology. They do not, however, give intact cell information because hemolysis is not an all or none phenomenon. Red blood cells empty themselves gradually so that, in a cell suspension considered 50% hemolyzed, there are both intact erythrocytes and cells which are incompletely lysed.

2. *Automated Serologic Dilutions* (26)

a. *Linear Dilutions:* In linear serial dilutions, concentration varies from tube to tube as an arithmetic progression. Thus, if C_1 is the concentration in

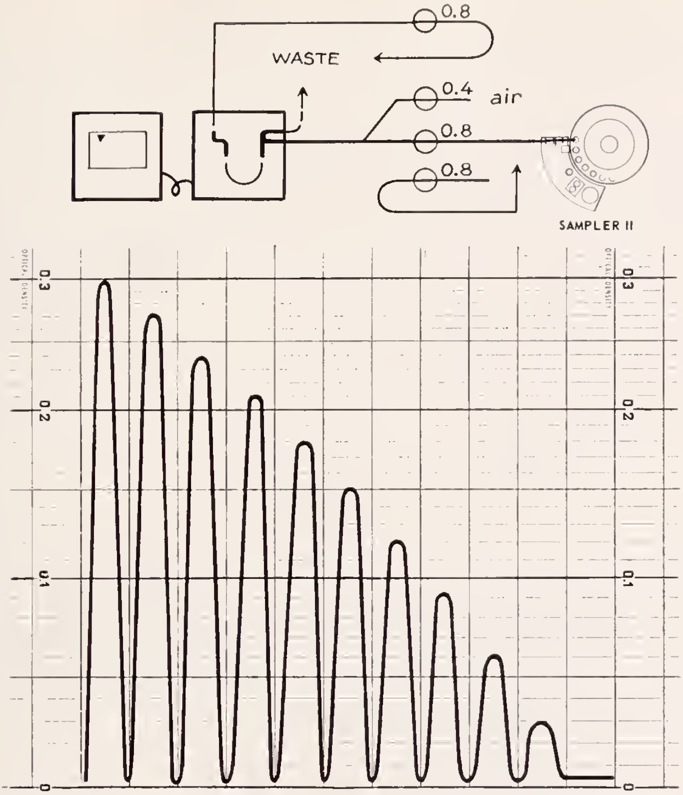

FIG. 2-1. *Turbidimetry and Beer-Lambert's Law.* Regularly decreasing concentrations are placed in sampler cups. A series of regularly decreasing peaks are recorded. The turbidimetric OD of 0.1% SRBC $\simeq$ 0.30. Cell-hemolysate mixtures can be prepared using cell suspension and precise hemolysate made isotonic with added concentrated buffer:

Tube Number	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Cell Suspension	5.0	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	0.0 ml
Hemolysate Solution	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0 ml

Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

tube No. 1, the concentration C_N in tube N is:

$$C_N = C_1[1 - (N - 1)r] \qquad \text{Equation 2-1}$$

Many procedures exist to make manual linear dilutions. This study will explore two—one semi-automatic, the other automatic.

Semi-Automatic: V_0 ml reagent at concentration C_1 is introduced into a receptacle (R). Two automatic pipettes deliver x ml and $2x$ ml, respectively. Then, $2x$ ml is taken from R and placed into cup No. 1 (concentration C_1), and x ml diluent is added to R. The new volume in R equals $V_0 - x$ and the concentration is:

$$C_2 = C_1(V_0 - 2x)/(V_0 - x) \qquad \text{Equation 2-2}$$

After thorough stirring of R, $2x$ ml is removed and introduced into cup

No. 2, (concentration C_2) and x ml of diluent is again added to R. The new concentration, C_3, is:

$$C_3 = C_2 \frac{V_0 - 3x}{V_0 - 2x} = C_1 \frac{V_0 - 2x}{V_0 - x} \times \frac{V_0 - 3x}{V_0 - 2x} = C_1 \frac{V_0 - 3x}{V_0 - x} \quad \text{Equation 2-3}$$

The same procedure is repeated for cups 4, 5, etc. In cup N, the concentration C_N will be

$$C_N = C_1(V_0 - Nx)/(V_0 - x) \qquad \text{Equation 2-4}$$

Equation 2-4 can be re-expressed as

$$C_N = C_1 \left[1 - (N - 1) \frac{x}{V_0 - x} \right] \qquad \text{Equation 2-5}$$

and as in Equation 2-1

$$r = x/(V_0 - x) \qquad \text{Equation 2-6}$$

Example:

To determine the dose of complement to be used for *CFR*, cups containing 2 ml ($2x = 2$ ml) of *GPS* diluted respectively, 1 %, 0.9 %, 0.8 % . . . must be tested. If each cup is filled with 2 ml of diluted serum, the value of x is 1 ml. Since the arithmetic progression is 0.1 (difference in concentration between two consecutive cups),

$$0.1 = \frac{x}{V_0 - x} = \frac{1}{V_0 - 1}; \quad \text{and} \quad V_0 = 11 \text{ ml}$$

Consequently, 11 ml *GPS* diluted 1 % are placed into R. 2 ml are aspirated with $2x$ pipette and placed into cup No. 1, and 1 ml of *VBS* is added to R with pipette x. The mixture in R is stirred thoroughly. The same steps are repeated for cups No. 2, 3, etc. until cup No. 10, at which time R is empty. R is empty when $(V - Nx) = 0$, which is at the eleventh cup.

The method of semi-automatic dilution is recommended for automated *CFR*. It is rapid; only 2 pipettes are needed. There is minimal contamination between successive cups, and the method provides reproducible titration curves. The values of parameters C_1, V_0 and x are determined by the serologist. Usually, the value for $(V_0 - x)$ is a multiple of 10.

Reverse Procedure:

Instead of obtaining serial dilutions of decreasing concentration, serial linear dilutions of increasing concentration can be made. Calculations similar to the above lead to the formula

$$C_N = C_0(N - 1)x/(V_0 - x) \qquad \text{Equation 2-7}$$

where C_N equals the concentration in cup N, C_0 represents the highest concentration to be obtained, and V_0 is the volume of diluent introduced into R at the start of the procedure. x is the quantity of reagent at concentration C_0 to be introduced into R at each stage of the technique.

Completely Automatic:

A concentration gradient decreasing in a linear fashion is illustrated in Figure 2-2. The concentration C_t at time t is calculated as follows:
When $t = 0$, volume is V_0 and concentration is C_0. At time t, volume is V_t and concentration is C_t.
The instrument samples a quantity of reagent, dm_t, between time intervals t and $(t + dt)$:

$$dm_t = (-2x)(C_t)(dt)$$

and

$$dm_t = d(V_t)(C_t) \quad \text{when} \quad V_t = V_0 - (x)(t)$$

hence

$$dm_t = d(V_0)(C_t) = V_t(dC_t) - (x)(d)(C_t) - (x)(d)(t)(C_t)$$

or

$$(V_0)(dC_t) - (x)(d)(t)(C_t) = (-2x)(C_t)(dt)$$

but

$$(V_0 - xt)(dC_t) = (-x)(C_t)(dt)$$

or

$$\frac{dC_t}{C_t} = -\frac{(x)(dt)}{V_0 - (x)(t)}$$

when $V_0 - xt = T$ and $dT = (x)(dt)$ the formula becomes:

$$dC_t/C_t = dT/T$$

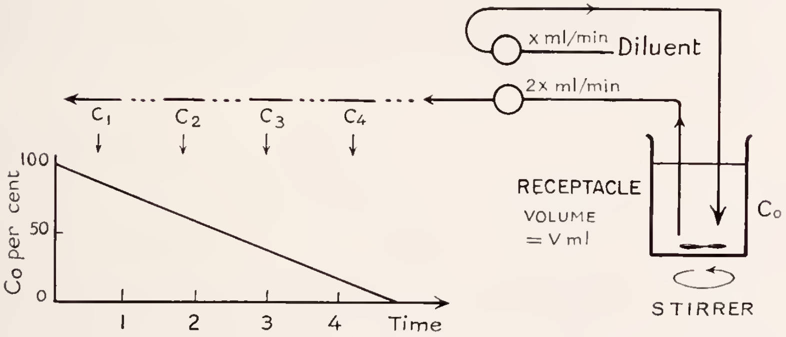

FIG. 2-2. *Automatic Continuous Linear Dilution.* Volume, V_0, of reagent at concentration, C_0, is placed into receptacle, R. Continuous sampling of 2x ml/min is accompanied by introduction of x ml diluent/min. C_1, C_2, C_3, C_4 $\cdots$ represent concentrations found at times t_1, t_2, t_3, t_4 $\cdots$
Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

This can be integrated from 0 to t: $\ln C_t/C_0 = \ln T/T$ or

$$C_t = C_0 \frac{T}{T_0} = C_0 \frac{V_0 - (x)(t)}{V_0} \qquad \text{Equation 2-8}$$

or

$$C_t = \left(C_0\, 1 - \frac{x_t}{V_0}\right) \qquad \text{Equation 2-9}$$

Equation 2-9 resembles Equation 2-5. The difference between them is that in Equation 2-5 the variable, N (number of tubes), is discontinuous while in Equation 2-9 the variable, t (time), is continuous. Figure 2-2 provides a decreasing linear concentration gradient. In the automatic procedure the order of tubes (No. 1, No. 2, No. 3) is replaced by the variable, time. Knowing time, t, the corresponding concentration C_t can be calculated.

Example:

A continuous complement titration can be performed to obtain a continuous dose-response curve on the recording paper. It is most convenient to make $x = 0.4$ ml/min, and to use two sampling probes, one removing 0.8 ml/min ($2x$), the other delivering 0.4 ml/min (x) into R. A 2% concentration, C_0, of *GPS* is chosen. V_0 is determined by the duration of the titration which ends when $t = V_0/x$ because R is empty. V_0 for a value of $x = 0.4$ ml/min is chosen from the following table:

Total Duration of Dilution	5 min	10 min	15 min	20 min
Initial Volume V_0	2 ml	4 ml	6 ml	8 ml

This procedure yields "dose-response" curves which are continuous and require no interpolation. Calculations are based on Equation 2-9 but a linear concentration scale can be substituted.

Reverse Procedure:

Using an increasing gradient from zero to C_0, volume V_0 of diluent is placed into R and $2x$ ml/min is removed from R while x ml/min of reagent of concentration C_0 is introduced into R. The formula of the linear dilution becomes:

$$C_t = (C_0)(x/V_0)(t) \qquad \text{Equation 2-10}$$

With $x = 0.4$ ml/min, $V_0 = 4$ ml, and $C_0 = 1\%$, we have the following concentrations as a function of time, t:

Duration of Dilution (min)	0	1	2	3	4	5	6	7
Value of C_t (%)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7

b. *Exponential Dilutions:*

Semi-Automatic: The conventional technique consists of making serial two-fold dilutions, the formula for which is

$$C_N = (C_1)(1/2)^{N-1} \qquad \text{Equation 2-11}$$

x ml of diluent is placed into all dilution tubes except tube No. 1 which receives $2x$ ml of reagent at concentration C_1. x ml is removed from tube 1 and mixed with the contents of tube 2, then x ml is removed from tube 2 and mixed with the contents of tube 3, etc. The geometric progression depends on $x/(V + x)$, and the dilution formula is:

$$C_N = (C_1)\left(\frac{x}{V + x}\right)^{(N-1)} \qquad \text{Equation 2-12}$$

Example:

Each tube should have a concentration equal to $\frac{1}{10}$ the concentration of the preceding tube ($r = 0.10$), and 1 ml is to be transferred from one tube to the next. Volume, V, is thus equal to 9 ml. For accuracy, calibrated pipettes can be used, but if the same pipette is used to perform the transfers, considerable contamination may take place.

Automatic:

The diluting system is that of Figure 2-2, except that x ml/min of the content of R is removed and x ml/min of diluent is introduced. During the time, Δt, $x\ \Delta t$ ml reagent at concentration C_t, $[x\ \Delta t(C_t)]$, is removed from a constantly maintained volume of V ml. The diminution of concentration, ΔC, is equal to:

$$\Delta C = \frac{(x)(\Delta t)(C_t)}{V} \quad \text{or} \quad \frac{\Delta C}{C_t} = -\frac{x}{V}\Delta t$$

and the differentials are

$$dC/C_t = -(x/V)\,dt$$

Integrating from 0 to t, $\ln C_t = -(x/V)t + \text{constant}$. The value of the constant is derived by making $t = 0$ and, hence, constant $= \ln C_0$. Therefore:

$$\ln C_t - \ln C_0 = -(x/V)t$$

$$\ln C_t/C_0 = -(x/V)t \qquad \text{Equation 2-13}$$

and

$$C_t = (C_0)e^{-(x/V)t} \qquad \text{Equation 2-14}$$

Table 2-1 lists the ratio C_t/C_0 for different values of x/V as a function of time, t. Knowing x = ml (input and output)/min., V = ml volume under change, and t = min., the concentration ratio, C_t/C_0, has been calculated from Equation 2-13

$$\ln C_t/C_0 = -(x/V)t$$

The technique resembles continuous linear dilutions, but numerous

TABLE 2-1

Automatic Exponential Dilution

Time t (minutes)	(x/V) Values											
	0.01	0.02	0.05	0.10	0.20	0.30	0.40	0.50	0.75	1.00	1.50	2.00
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1	0.99	0.98	0.95	0.90	0.81	0.74	0.67	0.60	0.47	0.36	0.22	0.13
2	0.98	0.96	0.90	0.81	0.67	0.54	0.45	0.36	0.22	0.13	0.05	0.01
3	0.97	0.94	0.86	0.74	0.54	0.40	0.30	0.22	0.10	0.05	0.01	
4	0.96	0.92	0.81	0.67	0.44	0.30	0.20	0.13	0.05	0.01		
5	0.95	0.90	0.77	0.60	0.36	0.22	0.13	0.08	0.02			
6	0.94	0.88	0.74	0.54	0.30	0.16	0.09	0.05	0.01			
7	0.93	0.87	0.70	0.49	0.24	0.12	0.06	0.03				
8	0.92	0.85	0.67	0.44	0.20	0.09	0.04	0.02				
9	0.91	0.83	0.63	0.40	0.16	0.06	0.03	0.01				
10	0.90	0.81	0.60	0.36	0.13	0.04	0.02					
11	0.89	0.80	0.57	0.33	0.11	0.03	0.01					
12	0.88	0.78	0.54	0.30	0.09	0.02						
13	0.87	0.77	0.52	0.27	0.07	0.02						
14	0.87	0.75	0.49	0.24	0.06	0.01						
15	0.86	0.74	0.47	0.22	0.05	0.01						
16	0.85	0.72	0.44	0.20	0.04							
17	0.84	0.71	0.42	0.18	0.03							
18	0.83	0.69	0.40	0.16	0.02							
19	0.82	0.68	0.38	0.14	0.02							
20	0.81	0.67	0.36	0.13	0.01							

combinations are possible by simply changing the value of V. The procedure of exponential dilution will be used to obtain model curves for kinetic studies.

Reverse Procedure:

V ml of diluent is placed into R. X ml reagent/min, concentration C_0, are introduced into R while x ml mixture/min are removed. The dilution formula is:

$$C_t = C_0[1 - e^{-(x/V)t}] \qquad \text{Equation 2-15}$$

c. *Special Type of Dilution:*

Automatic dilutions for a continuous flow system can be constructed using only residual material in the pump lines ("wash") (27). The principle of transfer from one sampler to another is shown in Figure 2-3.

Serum No. 1 is placed in the first cup of the first sampler. The probe removes serum No. 1 for 5 seconds (0.1 ml of serum). The probe then removes 0.5 ml of diluent. The two (0.1 ml of serum and 0.5 ml of diluent) are delivered into cup 1′ of the second sampler. The serial dilution in cup 1′ should equal 1/6, but in reality it is only 1/7 because some of the serum adhered to the walls of the pump line. Diluent instead of new serum is

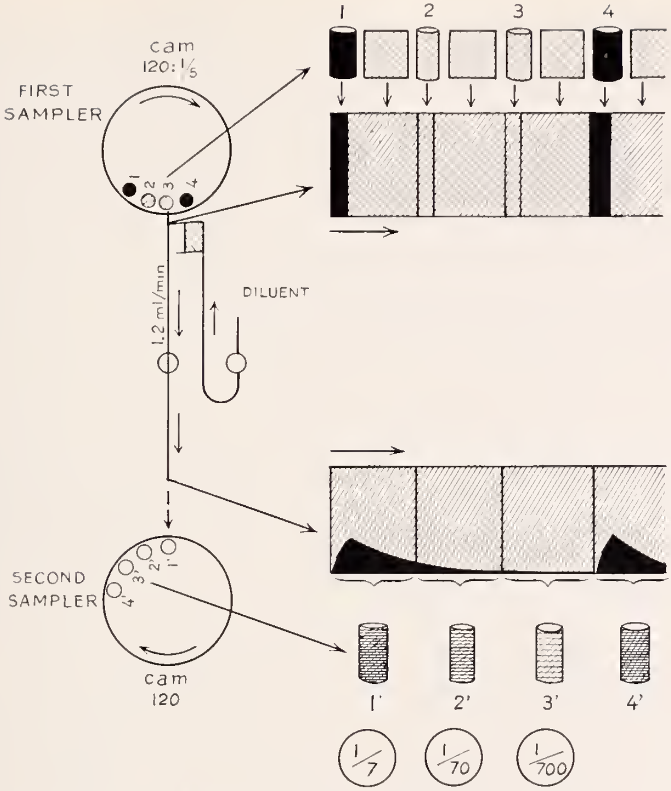

FIG. 2-3. *Schematic Representation of Automatic Dilution.* (see text)
Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

placed into cup 2 of the first sampler. Cup 2′ of the second sampler will receive 0.6 ml of diluent contaminated by the serum that adhered to the tube walls and the serum dilution in 2′ will be 1/70. Finally, cup 3′ will contain a dilution of about 1/700. Another serum which is placed into cup 4 of the first sampler will be diluted in cups 4′, 5′ and 6′ of the second sampler in the same manner.

The method is versatile. Many varieties of dilutions can be made merely by changing the time of sampling of the first sampler and by altering the delivery size of pump lines. Actual dilutions, however, should be assayed at least once by colorimetry.

3) *IMMUNE HEMOLYSIS STUDIED BY AUTOANALYZER*

1. *Kinetic Studies of Immune Hemolysis*

Immune hemolysis is the destruction of *RBC* by coupled hemolysin (or *A*) and *C*. It is the final phase of tests for *CF*. Kinetic analyses of immune hemolysis provide data on performance of the instrument and, thereby, suggest improvement in conditions for performing immune hemolysis.

Figure 3-1 gives a flow diagram and the kinetic curves of hemolysis obtained with different concentrations of *C*. *V* ml of *EA* are placed into *R* and maintained at 37°C. After *C* is added at zero time, the mixture is sampled continuously and assayed for lysis. The record shows the hemolytic curve as a function of time. The uppermost curve in Figure 3-1 is composed of five phases. (1) Phase of latency, which lasts from 45 seconds to 6 minutes. (2) Phase of increasing hemolysis. (3) Phase of maximal hemolysis. (4) Phase of decreasing hemolysis. (5) Final phase of no further hemolysis because either all cells have been destroyed or complement activity has been consumed.

a. *Effect of Complement Concentration on Immune Hemolysis:* If concentration of *EA* is kept constant while concentration of *C* is varied, a family of curves is obtained (Fig. 3-1).

b. *Titration of Complement* (kinetic method): A mixture of *E* and *A* is made in *VBS* 2+ so that the final concentrations of *RBC* are 10^7/ml and *A* is 1/2000. Five ml aliquots of this *EA* suspension are maintained at 37°C. At zero time for each, 0.1 ml of fresh human serum is added and the rate of hemolysis is determined. The time required for 50% lysis is estimated from the record. Table 3-1 shows these values and their relation to com-

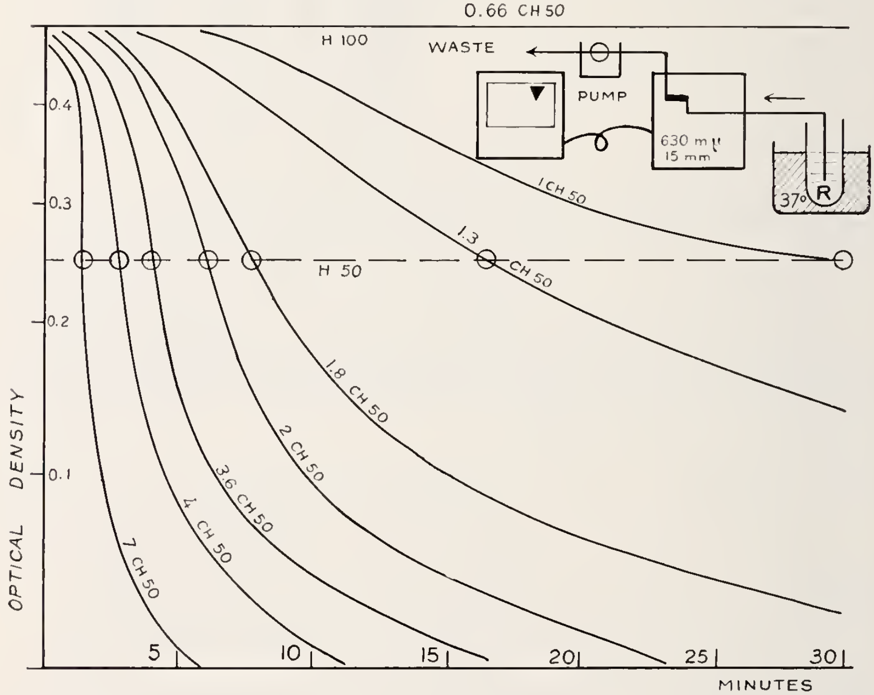

Fig. 3-1. *Kinetic Hemolytic Curves Obtained with Different Concentrations of Complement.* Sensitized erythrocytes, *EA*, are placed into receptacle, *R*. A given amount of complement is added at time zero. The recorder provides a family of sigmoidal curves for each quantity of complement. Each curve shows a period of latency, a phase of rapid hemolysis, and a phase of decreasing rate of hemolysis. Time required for 50% hemolysis (tH50) can be found for each curve.

plement units in the tested serum. This kinetic titration of complement can be semi-automated.

c. *Effect of Anticomplementary Substances* (*AC*) (Fig. 3-2): The flow diagram of Figure 3-1 is used. *EA* and *AC* are placed into *R* and, at time zero, 5 CH50 units of complement are added. The kinetic curves are studied relative to the control curve, *T*, obtained without the addition of *AC*. *Curve 1:* The addition of *AC* slows the onset of hemolysis without altering the shape of the curve. This is the effect of competition between *EA* and *AC* for complement. *Curve 2:* The addition of *AC* deforms the curve. The time for 50% hemolysis is the same as for curve 1, but the period of latency resembles that of control curve *T* and hemolysis is incomplete. *AC* is, therefore, antihemolytic.

2. *Analysis of Kinetic Curves of Immune Hemolysis*

The precision of kinetic curves provided by AutoAnalyzer permits mathematical analysis of results.

a. *Immune Hemolysis as a First Order Reaction:* In 1905, Henri (28, 29)

TABLE 3-1

Relation between Time Needed for 50% Lysis and Units of Complement Introduced

Time for 50% Hemolysis (min.)	CH50 Complement Units
30 min.	1.0
10 min.	1.5
6 min.	2
3 min. 45 sec.	3
3 min.	4
2 min. 30 sec.	5
2 min. 15 sec.	6
2 min.	7
1 min. 30 sec.	10
1 min.	15

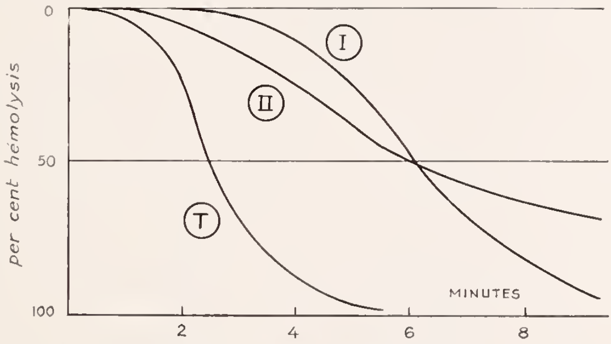

Fig. 3-2. *Effect of Anticomplementary Substances on Kinetics of Immune Hemolysis.* (see text)

Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

studied the speed of hemolysis of chicken red blood cells and derived the two following laws. (1) Speed of hemolysis is independent of the quantity of *RBC* provided the quantity of serum and the volume of liquid are kept constant. (2) At the beginning of the experiment there is latency, but the second part of the curve (phases 3, 4, and 5) is similar to a monomolecular reaction expressed by the equation

$$\ln a_c/(a_0 - a_t) = kt \qquad \text{Equation 3-1}$$

where a_0 = number of red cells at time zero, a_t = number of red cells at time, t, and k = rate constant of the reaction. Henri indicated similarities between enzymatic reactions and immune hemolysis, and Ponder (30–32) further developed this fundamental equation. Henri's equation was applied to the curve of Figure 3-1 obtained with a concentration of 1.8 CH50 units per ml. If the value of $k = 0.1$ min^{-1}, experimental and calculated values are given in Table 3-2. Henri's interpretation is thus only an approximation.

b. *Kinetic Law in a Heterogeneous Medium (33):* Proust and Tompkins' law is:

$$\ln \alpha/(1 - \alpha/2\alpha_i) = kt + \text{constant} \qquad \text{Equation 3-2}$$

α represents progress of the reaction (i.e., the amount of hemolysis), t = time, α_i is the point of the curve when rate of hemolysis becomes maximal. The law has been applied to the kinetics of immune hemolysis. One experiment gave the results shown in Table 3-3. Proust's law is linear for values of t between 120 and 350 seconds.

The preceding calculations indicate only that an equation can be fitted

TABLE 3-2

Henri Equation Applied to Curve of Fig. 3-1

Experimental OD	0.25	0.20	0.16	0.13	0.11	0.10	0.08	0.06	0.05
Calculated OD	0.25	0.21	0.18	0.15	0.12	0.11	0.09	0.06	0.05

TABLE 3-3

Proust Equation Related to Degree of Lysis

Time (seconds)	Optical Density	Degree of Lysis (α)	$\ln \alpha/[1 - (\alpha/2\alpha_i)]$
0	0.550	—	—
120	0.540	0.018	−4.00
180	0.515	0.063	−2.70
240	0.428	0.222	−1.26
270	0.350	0.364	−0.57
300	0.235	0.536	0.12
350	0.150	0.725	0.92
380	0.070	0.870	1.76
420	0.023	0.960	2.78

Maximal hemolytic rate α_i, is 0.510.

to a segment of a sigmoidal curve. Three hypotheses attempt to explain why kinetic curves are sigmoidal. The third is the one most widely accepted today.

Ponder (32) suggested that lysis takes place on a heterogeneous substrate with each sensitized red blood cell having its own fragility. The most fragile *RBC* are destroyed rapidly followed by the normal cells and lastly by the resistant ones. There is no evidence to support this view.

Alberty and Baldwin (34) proposed a "multiple hit" theory in which cells were equally sensitive but the number of lesions, S^*, varied among cells. *RBC* that received the most "hits" were destroyed more rapidly than the others. Alberty drew a model curve identical to experimental curves.

Mayer's hypothesis (35) is the "single hit" theory; i.e., if the cell is hit, S^*, it will be destroyed. Latency corresponds to fixation of the 9 components of complement. This "single hit" hypothesis can be used to evaluate the speed at which hemoglobin molecules leak from the cell. The volume of the empty cell, V, is close to 450×10^9 nm³. A "hit" produces a "hole" with an area of 2×10^2 nm². The flow, x, out of the "hole" is 2×10^2 multiplied by rate, 1, of outflow. The loss of hemoglobin follows the formula given in the study of exponential dilutions:

$$C_t = C_0 e^{-(x/V)t} \qquad \text{Equation 3-3}$$

A CH50 unit of complement removes 50% of cellular hemoglobin in 1800 sec. at 37°C. Therefore:

$$t1/2 = 1800 = \frac{0.69}{x/V} = \frac{0.69V}{x} = \frac{0.69 \times 450 \times 10^9}{200 \times 1}$$

hence rate of lysis $= 9 \times 10^5$ nm/sec $= 0.9$ mm/sec.

3. *Consequences of Kinetic Studies*

Figure 3-1 indicates that degree of hemolysis is a function of quantity, complement and time. The line of the abscissa, $t = 6$ min., cuts experimental curves at different ordinate values. With a rigorously controlled instrument, complement need not be completely consumed. Thus, the hemolytic phase can be abbreviated from the usual 30 or 45 min. at 37°C, to six min. at 37°C; this provides rapid and exact results when duration of the hemolytic phase is mechanically controlled. In the AutoAnalyzer, duration of the hemolytic phase is defined by the internal volume of the mixing coils and the flow delivery.

With duration of hemolytic reaction fixed at 6, 7 or 8 min., the unit of complement must be redefined. Here, it is preferable to use the term "minimal hemolytic dose" (*MHD*) and reserve the term "unit of complement" for physiopathologic data. The concept of *MHD* is more convenient though less precise for complement fixation reactions. One *MHD* is the minimal quantity of *GPS* that yields total lysis in a given experimental system. One is free to choose a preferred hemolytic system (concentration and quality of both *SRBC* and hemolysin, and whether or not *EA* is preincubated); one can also choose duration and temperature of the hemolytic reaction.

4. *Factors Affecting Immune Hemolysis*

a. *Optimal Temperature:* Figure 3-3 gives the flow diagram for this study. The concentration of *GPS* was chosen to produce 70% hemolysis at 37°C. At the start of the experiment the water bath was at 20°C, and a thermostatically controlled heater raised this temperature 1°C per minute until 55°C was obtained.

The resulting curve is U-shaped with optimal hemolysis at about 42°C. Below 30°C, hemolysis was negligible in six minutes (duration of the hemolytic reaction); thus 37°C is not a special temperature,

These results differ from those of Leon (36) who found optimal temperature for immune hemolysis close to 30°C. The duration of the hemolytic reaction explains the discrepancy between results. At 42°C, immune hemolysis occurs rapidly but complement activity is quickly lost; at 30°C, immune hemolysis takes place slowly but complement activity is much more stable.

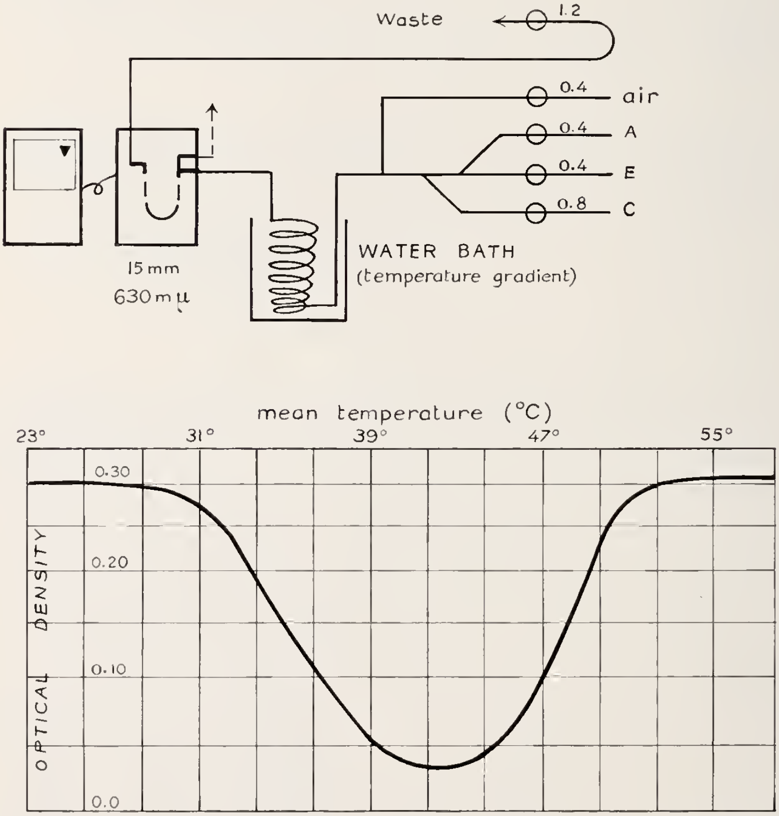

FIG. 3-3. *Automatic Determination of Optimal Temperature for Immune Hemolysis.* The flow diagram indicates factors that remain constant (antibody, erythrocytes, complement, duration of time for hemolysis). Temperature is changed from 23°C to 58°C in a linear manner. Optimum activity is obtained at 42°C.

Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

b. *Preincubation of Sensitized Erythrocytes:* In the flow diagram shown in Fig. 3-3, there is no preincubation of E with A. It is preferable to allow E to incubate with A and obtain an EA complex before adding complement. To demonstrate the effectiveness of preincubation, the instrumentation of Figure 3-3 can be used, but the water bath temperature is kept constant at 37°C, and only enough C for 30% lysis is used. After the recorder indicates constant and regular 30% lysis, 20 ml of E and 20 ml of A are mixed, and the mixture is immediately aspirated by both E and A pump lines. In three to four minutes, a new level of 65% hemolysis is attained. Thus, the same concentration of complement is more effective with presensitized

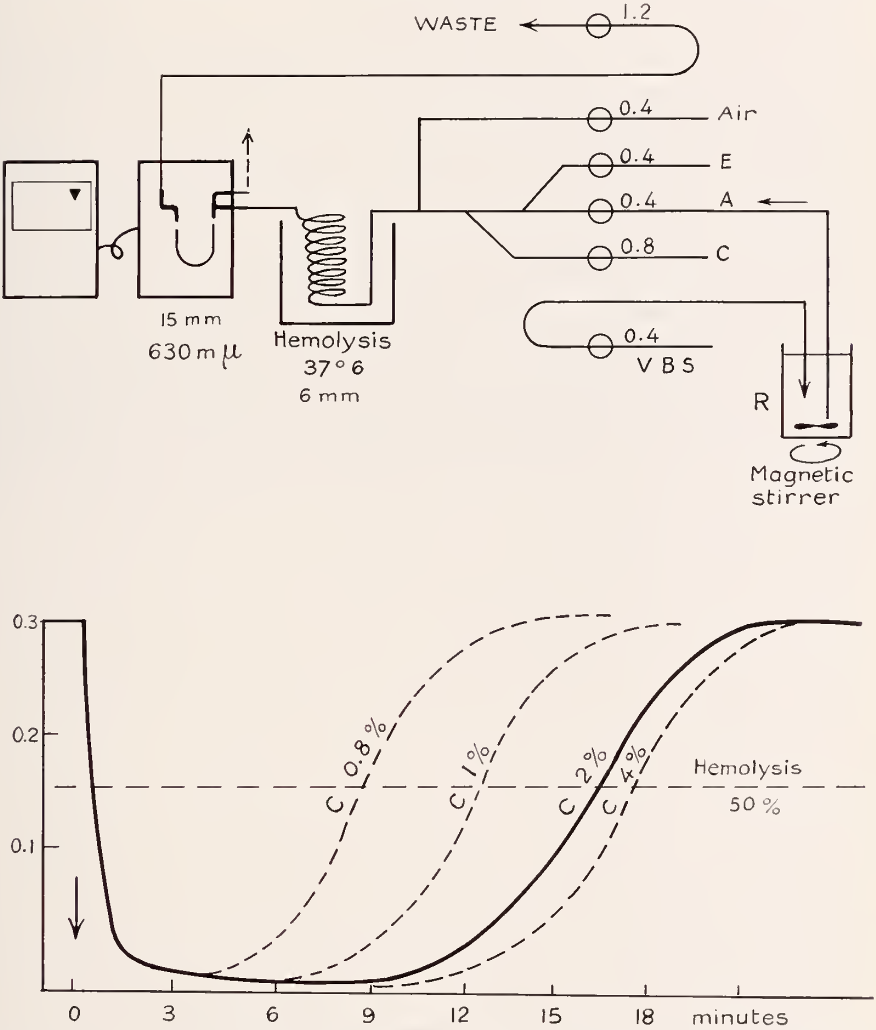
WASTE
1.2
0.4
Air
0.4
E
0.4
A
0.8
C
0.4
VBS
15 mm
630 mμ
Hemolysis
37° 6
6 mm
R
Magnetic stirrer
0.3
0.2
0.1
C 0.8 %
C 1 %
C 2 %
C 4 %
Hemolysis
50 %
0
3
6
9
12
15
18
minutes

FIG. 3-4. *Titration of Hemolytic Serum.* Top: The flow diagram indicates how hemolytic serum in receptacle, *R*, is automatically diluted by continuous addition of buffer. Bottom: Titration curves represent degree of hemolysis as a function of dilution time.
Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

EA than with separate *E* and *A* reagents. Mixing coils can therefore be introduced for the purpose of preincubation of *E* and *A*.

5. *Automatic Titration of Hemolytic Serum*

The flow diagram of Figure 3-4, with exponential dilution, is used for titration of *A*. Two ml of *A*, diluted 1/1000 in *VBS* 2+, is placed into *R*. The dilution formula is:

$$C_t = 1/1000\ e^{-0.2t}$$

since $x/V = 0.4/2$ it is equal to 0.2. The experiment lasts 20 min. At that moment, C_{20} equals 1/100,000 (see Table 2-1).

GPS diluted to 2% yields a sigmoidal curve. Other concentrations of complement provide a family of sigmoidal curves that indicate a quantitative relationship between *A* and *C;* as the quantity of *C* decreases, more *A* is needed to lyse the same number of *RBC* (Fig. 3-4).

Concentration of *A* is chosen according to its lytic strength and its agglutinating property. Agglutination interferes with lysis and must therefore be avoided. It is best to use an excess of *A* approximately four to six times the minimum hemolytic concentration (*MHC*).

AutoAnalyzer instrumentation is useful for the study of all hemolytic systems, and several flow diagrams have been given for such work. The kinetics of hemolysis of human *RBC* by streptolysin *O* resembles that of immune hemolysis (37–40). On the other hand, other bacterial hemolytic systems such as those of alpha streptolysin (41), Clostridium perfringens, lecithinase, or pneumococcus lysin (unpublished data) seem to act like simple enzymes giving first order kinetic curves.

Addendum

In the November–December 1973 issue of *The Mount Sinai Journal of Medicine,* Dr. Allan E. Kark and I reported on "The Role of Splenectomy in the Management of Left Subphrenic Abscess." It has recently been brought to my attention that when the patient described was presented at a surgical staff conference, Dr. Ralph Colp, Emeritus Professor of Surgery and long-time Surgeon to The Mount Sinai Hospital, was present, and suggested that splenectomy be employed in the management of the patient. This suggestion was based on Dr. Colp's work in the therapy of Ludwig's angina in the preantibiotic era. Dr. Colp had demonstrated that only removal of the blocking submaxillary gland would allow drainage of the cellulitis around it. By analogy, he suggested splenectomy in the management of our patient.

Our failure to acknowledge Dr. Colp's suggestion was completely inadvertent and we regret the omission. As pointed out in the article, the use of splenectomy was crucial to the eventual good result.

Arthur H. Aufses, Jr., M.D.

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COMPARATIVE ANTIBIOTIC SUSCEPTIBILITY OF MICROORGANISMS ISOLATED AT MSH IN 1973

Gram Positive Organisms	DRUGS																
	Ampicillin	Carbenicillin	Cephalothin (Keflin)	Chloramphenicol (Chloromycetin)	Erythromycin—Oleandomycin*	Gentamicin	Lincomycin Clindamycin	Neomycin—Kanamycin*	Penicillin	Oxacillin (Prostaphlin) Methicillin (Staphcillin)*	Polymyxin—Colymycin*	Streptomycin	Tetracycline	Vancomycin	Sulfonamide	Nalidixic Acid (Neggram)**	Nitrofurantoin (Furadantin)**
Staphylococcus aureus	3	—	1	2	2	—	1	1	4	1	—	—	2	1	4	—	—
Strept. faecalis (Enterococcus)	2	—	5	2	4	—	6	5	2	6	—	—	5	2	6	—	—
Beta Hemolytic Streptococcus	1	—	1	1	1	—	1	6	1	1	—	—	3	1	6	—	—
Streptococcus viridans	1	—	1	1	1	—	1	5	1	2	—	—	3	1	5	—	—
Anaerob. and Microaeroph. Streptococci	1	—	1	1	2	—	2	5	1	2	—	—	2	1	6	—	—
Diploc. pneumoniae (Pneumococcus)	1	—	1	1	1	—	2	6	1	1	—	—	2	1	3	—	—
Clost. perfringens (B. Welchii)	1	1	2	1	1	5	3	6	1	3	—	—	3	—	6	—	—

Gram Negative Organism																	
Bacteroides Species	4	1	3	1	2	4	2	5	4	5	—	5	3	—	5	—	—
Esch. coli	3	2	3	2	—	1	—	2	—	—	2	3	4	—	4	1	1
Enterobacter (Aerobacter) aerogenes	5	3	5	3	—	1	—	2	—	—	2	2	4	—	4	1	1
Klebsiella Species	5	6	2	3	—	1	—	1	—	—	2	2	5	—	5	1	1
Indole Positive B. proteus	4	2	4	1	—	1	—	1	—	—	6	2	4	—	4	1	3
Proteus mirabilis	2	1	2	2	—	1	—	1	—	—	6	2	5	—	4	1	3
Ps. aeruginosa (B. pyocyaneus)	6	2	6	5	—	1	—	2	—	—	2	3	6	—	4	5	6
Pseudomonas Spp.	5	4	6	4	—	2	—	3	—	—	3	4	5	—	4	4	6
Salmonella	2	1	2	2	—	1	—	1	—	—	2	2	3	—	5	1	1
Serratia marcescens	6	5	6	5	—	1	—	3	—	—	5	4	5	—	5	3	3
Shigella	3	—	1	2	—	2	—	2	—	—	1	3	4	—	5	1	1
Haemophilus influenzae	2	2	2	1	1	1	4	2	2	3	—	1	2	3	1	—	—
Mima-Herellea Group	3	2	5	3	—	1	—	1	—	—	2	2	2	—	3	1	5

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1. over 95% strains susceptible
2. 76–95% strains susceptible
3. 60–75% strains susceptible
4. 40–59% strains susceptible
5. 10–39% strains susceptible
6. less than 10% strains susceptible

* Equivalent Spectra of Activity

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Illustrations, charts, or tables must be so arranged that they are comprehensible to the reader without reference to the text. Legends should be typed on a separate sheet at the end of the paper. Manuscripts will be held for two months after publication. They will then be returned to the author on request or will be destroyed.

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ARTHUR J. BENDICK
1888–1973

In Memoriam

ARTHUR J. BENDICK, M.D.

1888–1973

Arthur J. Bendick died on October 26, 1973.

After graduation from City College, Arthur received his M.D. degree from Columbia College of Physicians & Surgeons in New York City. He interned at The Mount Sinai Hospital, New York, in 1909, and thus began a medical career at Mount Sinai which spanned his entire professional life. Upon his retirement, he was Consultant Radiologist and Clinical Professor Emeritus at Mount Sinai School of Medicine.

As an eminent academician and clinical practitioner of Radiology (and he was one of the first in this specialty), he served in three major hospitals, Mount Sinai, Montefiore, and Beth Israel, each affiliated with the Federation of Jewish Philanthropies. At Montefiore and Beth Israel, he was Director of Radiology. He joined Beth Israel Hospital in 1942, and remained until 1955. At all three hospitals he was most influential in the development of high quality professional and teaching standards for Radiology Departments in metropolitan voluntary hospitals. At the same time he pioneered technical advances in the private practice of radiology, and was active in many professional societies including the Radiological Society of North America. His outstanding reputation earned for him the Presidency of the New York Roentgen Society in the early 1950's.

Dr. Bendick is well known for his original description of the radiological diagnosis of the azygos lobe on the routine chest film. Perhaps not so well known to some, he made a most important contribution in the technical aspects of the Rubin test for tubal patency. He was the author of a monograph "Diagnostic Advances in Gastro-Intestinal Radiology." His numerous publications include original research in hysterosalpingography, gastrointestinal fluoroscopic studies, radiologic diagnosis of pulmonary tuberculosis, and the radiologic follow-up of pulmonary tuberculosis treatment prior to the discovery of drug therapy.

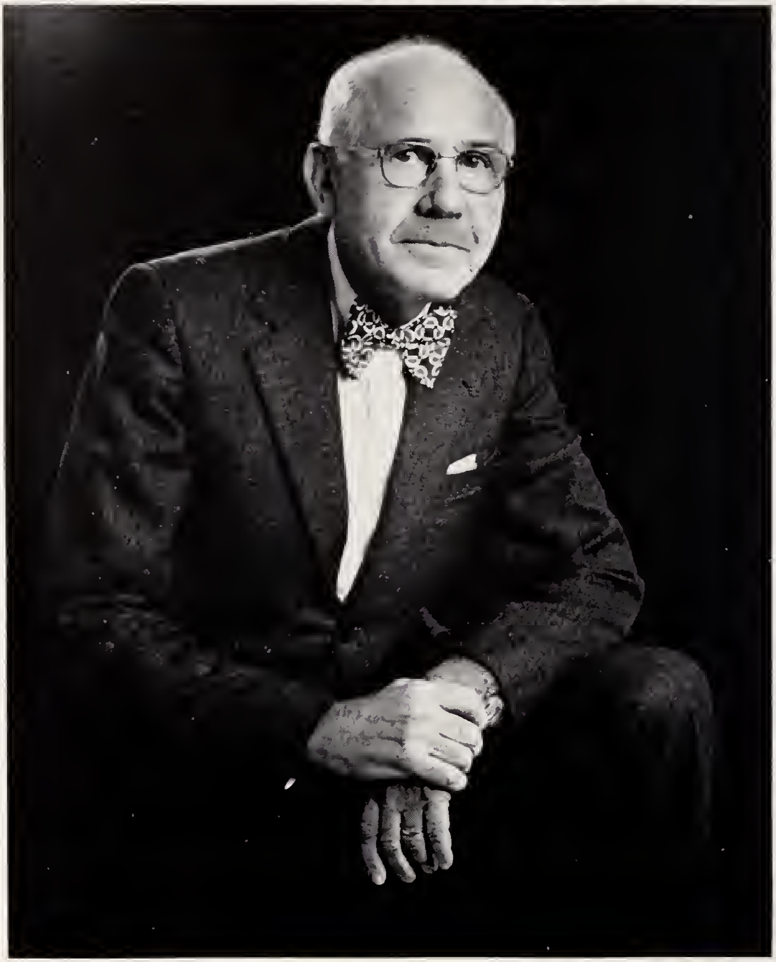
Dr. Bendick's long life spanned several generations and he participated in some of history's great events. When the survivors of the sinking of the Titanic arrived in New York in 1912, he served as ambulance driver, bringing that disaster's casualties to Mount Sinai. In World War I he was a captain with the Mount Sinai Hospital Unit, Base Hospital #3 A.E.F. in France. Twenty years later, one of his fellow officers reminisced: "While over there he played with his electrical toys all day and still found time to act as Sanitation Officer." In those days radiology was quite young, and Dr. Bendick had to depend upon his own mechanical and technical ability to perform radiology of significance. Having contracted tuberculosis while in military service, he spent a year at a ranch in Colorado where his love of the outdoors was enhanced. Medically ineligible for service in World War II, he was among those physicians who provided high quality professional care for the civilian population. His special interest in the care of military personnel was rekindled when he joined the Veterans Administration (after his "first retirement" from hospital and office practice) at the Veterans Hospital at Castle Point, N.Y., a position he held with distinction for ten years before retiring a second time in 1965. While there, he contributed significantly to the care of Korean War veterans and the early casualties of the Vietnam War. After this second retirement, he returned to Beth Israel Hospital as part-time Consultant and remained active even after his 80th birthday.

Dr. Bendick's steadfast commitment to his patients, students, and colleagues was more than equaled by his devotion to family. To his cherished wife, Marcelle, and two brothers and two sisters, he lavished affection, concern and caring. To his nephews, Mark and Robert Bendick, he was confidante and mentor.

His vigor and vitality were suddenly and sadly diminished by an unfortunate accident in Florida, where he and his beloved Marcelle were to have lived in true retirement. He never recovered from that event, although he showed great inner strength in his effort to regain his previous physical status. Almost a year later, complications from that accident finally overcame the mighty and vigorous Arthur Bendick in a peaceful death. Throughout the last year, his dear Marcelle, his companion through life, was at his bedside continuing the beautiful relationship that was always evident between Marcelle and Arthur during their 42 years of wedded life.

The Boards and the Staffs of the Mount Sinai Medical Center and of the Beth Israel Medical Center, especially the Departments of Radiology of both institutions, were deeply grieved at the passing of Arthur J. Bendick. We shall all remember him (as expressed by Dr. Bernard S. Wolf) as—"a pioneer radiologist and scholar, a dedicated physician and teacher, a gentleman and a gentle man."

Abraham Geffen, M.D.
for the
Editorial Board



ARTHUR M. MASTER
1895–1973

In Memoriam

ARTHUR M. MASTER, M.D.

1895–1973

Arthur Master's death on September 4, 1973 at the age of 77 ended the long productive career of one of this country's renowned cardiologists.

In 1933, Arthur Master at the age of 38 was appointed Chief of the Electrocardiography Department at The Mount Sinai Hospital. At that time cardiology was first being recognized as an important specialty and new techniques were being introduced into the clinical study of cardiac patients. Arthur Master was destined to be in the forefront of some of the major cardiological advances in the next four decades.

After graduating with a distinguished record from City College and Cornell University Medical College, and interning at Mount Sinai from 1921 until 1923, Master was awarded a Cornell Traveling Fellowship in Sir Thomas Lewis's laboratory in London. His article with Lewis on heart block and the supernormal recovery phase of A-V conduction remains a frequently quoted classic.

It was not long after his appointment at Mount Sinai that Master rose to the forefront in clinical practice, teaching, and research in cardiology. His intense drive, dynamism, inventiveness, eagerness, flow of ideas and enthusiasm spurred us all to carry out research on many facets of clinical cardiology. Studies at the patient's bedside, in the cardiac clinic, and in the cardiographic laboratory produced data on electrocardiography, noninvasive graphic tracings, circulation time and venous pressure, cardiac output (acetylene method), and cardiac fluoroscopy and roentgenkymography. Despite his busy consultation practice, he became personally involved in every project carried out in his department, from the writing of the original protocol to the gathering and analysis of clinical data and the actual writing of the paper. The result was the greatest outpouring of original reports and papers from the Division of Cardiology that Mount Sinai had ever seen—a total of approximately 440 published articles and five textbooks bearing his name and those of his staff.

There are two particular areas in which Master's research contributed to major advances in our knowledge—namely, our understanding of coronary artery disease and development of techniques for stress testing of the heart. Modern sophisticated research techniques are now confirming many of the observations made 30 years ago by Master and his associates. At that time it was evident to us that heart failure and shock were the two factors which affected the prognosis of acute coronary thrombosis. The survival of patients with pump failure and cardiogenic shock in myocardial infarction has not improved much since then. All aspects of myocardial infarction were studied by Master and his students, including prevalence, risk factors, the electrocardiogram, cardiac arrhythmias, abnormal heart sounds and gallop rhythms, abnormal left venticular contraction detected by fluoroscopy and roentgenkymography (presently referred to as dyskinesia), treatment, rehabilitation, and follow-up. These are all problems which are currently under active clinical investigation.

Another aspect of coronary artery disease associated with Master's name is acute coronary insufficiency. His concept of acute myocardial ischemia and anoxia in the absence of acute coronary occlusion now forms the basis of our present concept of ischemic heart disease and our present approach to the management of patients with unstable angina, crescendo or "preinfarction" angina, and "impending" myocardial infarction. Also, Master's emphasis on statistical methods of follow-up of postinfarction patients resulted in the complete reversal of the prevailing attitude that such patients are completely disabled and should retire from their work. His optimism concerning the degree of recovery and longevity has been fully confirmed by current studies on the life history of coronary artery disease.

The second major area of Master's scientific contribution was the exercise stress test. This was based on his two-step test of cardiac function which he had introduced with Enid Oppenheimer in 1925. In the late 1930's Master assigned Rudolph Friedman and myself to study the effect of his test on the electrocardiogram. After two years of intensive study we convinced him that the future of the exercise test rested on the demonstration of an ischemic electrocardiographic change rather than its effect solely on blood pressure and heart rate. Since publication of our first report in 1942, Master's continuing analysis and correlative study of many thousands of two-step exercise tests made this test, which bears his name, the most practical office and hospital procedure for the evaluation of patients with angina pectoris. Not even the newer sophisticated techniques of coronary arteriography and maximal treadmill and bicycle ergometer stress tests have displaced the two-step test from the daily practice of cardiology. The fruits of this test alone should assure him his place in cardiologic history.

Arthur Master received many honors in his lifetime. He was a past president of the New York County Medical Society and of the American College of Chest Physicians, and held high office in the New York and American Heart Associations, the American College of Cardiology, the New York Academy of Medicine, and other prestigious medical societies. He served on the editorial boards of several medical journals, including *Chest* and the *New York State Journal of Medicine*. His most cherished title was that of Attending Cardiologist of The Mount Sinai Hospital, a position he held with great honor and distinction until 1957 when he became Consulting Cardiologist. In 1968, with the founding of the Medical School, of which he was a sponsor, he was appointed Clinical Professor Emeritus of Medicine. Master's greatest legacy to cardiology and to The Mount Sinai Hospital is the long list of renowned cardiologists who received their training and inspiration in his department. He left an unforgettable mark on all who worked with him. It is, therefore, fitting that in his will he established the Arthur Master Chair in Cardiology which will perpetuate his influence on the teaching and practice of cardiology at The Mount Sinai Hospital.

SIMON DACK, M.D.
for the
EDITORIAL BOARD

ARTHUR S. W. TOUROFF
1900–1973

In Memoriam

ARTHUR S. W. TOUROFF, M.D.

1900–1973

Dr. Arthur S. W. Touroff, Director Emeritus of the Department of Surgery, Consultant Surgeon to The Mount Sinai Hospital and Professor Emeritus of Surgery, died at home September 10, 1973 at the age of 73. From the time of his college days as an outstanding student and all-round athelete to his final arrival as a leader among his fellow surgeons, one was impressed by his quiet drive for perfection and his consideration for the younger surgeons who learned from him and were given ample opportunity to develop and grow under his guidance. To have known "Artie" or "Sig" as he was called by his friends and intimate colleagues was to have admired and loved him. I will not attempt to enumerate his published papers or the numerous honors bestowed upon him by both national and international surgical societies. He was a founder and first president of The New York Society for Cardiovascular Surgery and helped found the first cooperative blood vessel bank in New York City in 1950. He had a first-name rapport with almost all leading surgeons and could and did open doors for surgical neophytes. He always tried to develop and nurture young talent. He not only had skill and courage, but could impart both these traits to the men around him.

After a well-rounded general surgical background, he gradually shifted his interests to thoracic surgery and eventually to cardiovascular surgery. He traveled extensively, attended surgical meetings throughout the country, and brought back new concepts which he immediately used and improved upon. With the advent of World War II, he volunteered early and received the Legion of Merit and Conspicuous Service Cross as director of thoracic surgery at a large army hospital in England. Cases were sent from the entire European theatre of operations to this center. He instituted many new operative procedures and developed a well-organized rehabilitation regimen for thoracic casualties.

He returned a colonel and soon was again completely involved in chest and cardiac surgery at Sinai. He was appointed as a full attending surgeon. Cases flowed in for treatment. Physicians and surgeons came from all over to see and hear what was new in chest surgery at Mount Sinai.

From patent ductus surgery he soon progressed to Blalock procedures, aneurysm resections, and vessel replacements with vein grafts, when a great tragedy occurred. He was stricken by an illness from which he never recovered. He ran the service from his sick bed in the hospital and by phone from his sofa at home where he was cared for by his loving wife and family with a devotion which he truly deserved. He never recovered sufficiently to assume the leadership for which he had worked so long and so hard.

I will always remember him just I as saw him on ward T in July 1934 when I, as a fresh surgical intern, was introduced to the great Dr. Arthur S. W. Touroff. It is difficult to project or prognosticate what might have happened if he had not suffered the tragic illness at the very pinnacle of his career. His drive, interest, and dedication to the development of the younger generation of surgeons were his greatest contribution.

Gabriel P. Seley, M.D.
for the
Editorial Board

JOSEPH TURNER, M.D.
1892–1973

In Memoriam

JOSEPH TURNER, M.D.

1892–1973

Dr. Joseph Turner died suddenly on July 6th, 1973 of a coronary closure in his eighty-second year. Despite previous cardiac attacks he was seemingly in good health and active until fatally stricken.

For over fifty years—the first twenty-five spent in an active role, the latter twenty-five as Consultant to the Board of Trustees—he was at the helm of the administration of The Mount Sinai Hospital. During this time his vision, his expertise and purposefulness did much to enhance the Hospital's stature and reputation.

Joseph Turner was born and educated in Philadelphia. He received his degree from Jefferson Medical College in 1915 and interned at Jewish Hospital, graduating as a House Physician. He served in World War I in the American Expeditionary Forces, attaining the rank of Captain. He was the recipient of the War Medal in recognition of his outstanding performance.

Upon return to civilian life his interest in hospital administration was immediately manifested and he became Medical Superintendent of Eagleville Sanitarium. He served there for three years until broader horizons beckoned and he came to Mount Sinai in 1922 as Assistant Director. This proved to be an opportunity the young Turner used well. Five years later he was elevated to Associate Director (1927).

During this period he was fortunate to serve under Dr. S. S. Goldwater, the outstanding and well-recognized authority in Hospital Administration and Planning. It is unnecessary to emphasize how much Turner absorbed in working with the Master and how well-prepared he was by reason of this preceptorship to assume the Directorship of the Hospital in 1928 when Dr. Goldwater resigned to become Health Commissioner of New York City.

The new Director followed in the footsteps of his predecessor and began a building program which established Mount Sinai on a par and in strong competition with the large Medical Centers. The erstwhile Pediatric Building and the Housman Pavilion, then known as Semi-Private, with its new eighth and ninth floor operating rooms are but two of the landmarks that issued from his drawing board. Until the present era, with modernization, the physical plant mirrored his expertise.

Dr. Turner served under Presidents Blumenthal, Arnstein, Kops, and Bernheim. with Turner's guidance, their dynamism and intense devotion to the best interests of the hospital were translated into initiating a policy of full-time Directors of the major services. Teaching and Research evolved as complementary to this policy. These were the "seedlings" of the beginnings of the Mount Sinai School of Medicine we witness today. I think it can be fairly said that "he was not the first by whom the new was tried nor yet the last to lay the old aside." His outstanding attributes were rectitude, fairness, honesty, and complete objectivity.

It was not long before Dr. Turner's reputation was established as a result of his publications. His knowledge was utilized as an instructor in hospital administration at Fordham University and as lecturer at Columbia and Northwestern Universities. He published significantly in the journals covering his special field, particularly in *Modern Hospital* and *Hospital Management*. He collaborated with Dr. S. S. Goldwater to write *On Hospitals* which was published in 1947 in book form.

He occupied many prestigious positions. He was Governor and Vice-President of the Greater New York Hospitals Association. He was the US Delegate to the International Hospitals Federation, attending meetings in London and Brussels. He was instrumental in promoting the organization of Blue Cross. He was consultant on hospital planning anad construction to several hospitals in the United States and abroad. He was a member of the American and International Hospitals Association as well as of the American Public Health Association.

His versatility never showed to better advantage than during World War II when he maintained a completely functioning hospital in spite of the loss of doctors, nurses, and personnel to the Armed Services. Particularly noteworthy was his recruitment for Base Hospital #3 which he saw activated in September 1942 and which served with distinction in North Africa, Italy, and Southern France. His personal interest was shown by a visit to Camp Rucker in Alabama where the Unit was staging before going overseas.

Joe Turner was gifted in many ways. He loved music and in his college days had taught himself to play the mandolin. A few years later he took instruction from a professional cellist and was soon playing chamber music with a Doctors' Quartet which met Sunday mornings at his home. A friend who collected fine string instruments lent them to Turner for several years, to be used in his home by professional musician friends who came frequently to play chamber music. It was my privilege to be invited to a number of these Saturday night musicales. The setting was memorable. The large reception hall of his home was reserved for the musicians with the guests seated almost in the manner of a theatre in the round. Even now it is difficult to decide whether the music or the sumptuous collation that followed, provided by his gracious wife, Henrietta, was the more appreciated and applauded.

When he became Consultant to the Board of Trustees in 1948 his versatility manifested itself anew. His penchant for music had been curtailed by a crippling arthritis of the small joints of his fingers but he had another "string attached to his bow."

Over the years he had frequently met with Mr. George Blumenthal whose beautiful home was a veritable art museum. With Mr. Blumenthal as his mentor, Turner became keenly interested anad knowledgable in the realm of the arts. He regularly vacationed abroad, visiting major museums anad viewing private collections. For years he had been making his travels a matter of photographic record and this hobby, almost by accident, developed into an absorbing avocation. A chance use of his Leica in the Louvre one day had produced transparencies of such beauty that he, to use his own expression, was "trapped."

He sought entrèe and received permission to photograph prestigious collections throughout Europe as well as this country and his collection of slides now runs into the thousands. They are borrowed by many lecturers at museums and universities and duplicates requested by leading museums throughout the world. He, himself, gave many private showings with talks enlivened by personal anecdotes to friends, cultural and church organizations, and community groups. The new-found hours of freedom in his "retirement years" were filled with activity as he polished his technical skills and advanced his interest into a unique and captivating avocation, one which brought him great personal satisfaction, broad worldly contacts, and a continued enjoyment of life, not too often the good fortune of a lesser spirit.

Dr. Turner leaves a devoted wife, affectionately known as Hetty, and two sons, one of whom followed him into the medical profession.

Those who were privileged to have known Dr. Turner will recognize that he made significant contributions to Mount Sinai and his memory will be revered by his many friends and acquaintances.

PERCY KLINGENSTEIN, M.D.
for the
EDITORIAL BOARD

Spontaneous Cerebellar Hematomas

SIDNEY A. HOLLIN, M.D., ROBERT E. DECKER, M.D., AND
SIDNEY W. GROSS, M.D.

Cerebellar hemorrhage occurs in less than fifteen percent of all cases of nontraumatic intracerebral hematoma (1–8). The majority of patients with cerebellar hematomas are hypertensive. If hypertension, large vascular malformations (over 2 cm in size), aneurysms, and blood dyscrasias are excluded, a much smaller group of "spontaneous" cases remains. This group, consisting of cerebellar hematomas of either unknown etiology or associated with small ("cryptic") malformations (9–13), is uncommon. In large series of cerebellar hemorrhage (1, 4, 5, 7, 8), such cases usually account for a relatively low percentage of the total.

The role of the small arteriovenous malformation in the production of cerebellar hematomas was emphasized by Odom et al (14). McCormick et al (12, 15) pointed out that "cryptic" posterior fossa arteriovenous malformations are probably not as rare as previously thought.

During the past ten years, seven patients with spontaneous cerebellar hematomas were treated at The Mount Sinai Hospital. In five cases a small arteriovenous malformation was found.

Case Reports

Case 1. A 35-year-old woman entered the hospital with a five-month history of headaches and vomiting. Her symptoms gradually progressed on severity. Shortly prior to admission she developed a clumsy gait and difficulty with balance.

General physical examination was normal.

On neurologic examination there was early papilledema. Her gait was ataxic. She tended to fall toward the left. The Romberg was positive. The rest of the neurologic examination was normal.

Routine laboratory studies were negative.

Skull and chest x-rays were normal. Pneumoencephalography demonstrated anterior displacement of the fourth ventricle with herniation of the cerebellar tonsils.

Suboccipital craniectomy was performed. A hematoma was evacuated from the vermis. No mural nodule or abnormal vessels were noted. Portions of the hematoma wall were biopsied, but no vascular malformation was found.

Postoperative course was uneventful.

Case 2. A four-year-old girl was admitted to the hospital because of headaches, vomiting, and dizziness of three weeks' duration. She had progressive difficulty walking and trouble with balance. The patient began to drag her right leg and fall toward the right.

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Requests for reprints should be sent to: Sidney A. Hollin, M.D., Department of Neurological Surgery, The Mount Sinai Hospital, Fifth Avenue and 100th Street, New York, N.Y. 10029.

General physical examination was normal.

On neurologic examination her head was tilted toward the left. There was early papilledema. Nystagmus was present on horizontal gaze toward either side. The patient walked with a broad-based gait and tended to fall toward the right. There was moderate intention tremor and clumsiness of the right extremities. The rest of the neurologic examination was normal.

Routine laboratory studies were normal.

Skull and chest x-rays were negative. Pneumoencephalogram demonstrated mild hydrocephalus with deviation of the fourth ventricle toward the left side.

Suboccipital craniectomy was performed. A large right cerebellar hematoma, consisting of wine-colored fluid, was evacuated. No abnormal vessels were noted. However, microscopic examination of biopsies taken from the hematoma wall revealed an angiomatous malformation.

Postoperative course was uneventful.

Case 3. A nine-year-old girl was brought to the hospital a few hours after the sudden onset of headaches, vomiting, and coma. Eleven months before she had a mild head injury with no loss of consciousness. However, shortly after the accident she became listless and unsteady and had difficulty holding objects in her right hand. The patient was admitted to another hospital where lumbar puncture revealed mildly xanthochromic cerebrospinal fluid. Her condition gradually improved spontaneously. She was asymptomatic for several months until the abrupt onset of her present illness.

On examination the patient was deeply comatose. Respirations were slow and irregular. There was only slight reaction to painful stimulation. Bilateral papilledema with hemorrhages was present. Corneal and oculocephalic reflexes were absent. Plantar reflexes were extensor.

Routine laboratory tests were normal.

Skull and chest x-rays were normal. Lumbar puncture revealed grossly bloody cerebrospinal fluid with elevated pressure.

The patient expired shortly after admission.

At autopsy a right cerebellar hematoma, which had ruptured into the fourth ventricle, was found. There was a small arteriovenous malformation in the hematoma wall.

Case 4. A 12-year-old girl entered the hospital with a five-week history of headaches, vomiting and dizziness. Three weeks prior to admission she began to have difficulty with balance and was observed to have a head tilt.

General physical examination was normal.

On neurologic examination her head was tilted toward the left. On palpation the skull overlying the left side of the posterior fossa was considerably more prominent than the right. The patient had a broad-based ataxic gait. The rest of the neurologic examination was unremarkable.

Routine laboratory studies were negative.

X-rays of the skull disclosed enlargement of the left side of the posterior fossa. Pneumoencephalography failed to fill the ventricles. Left brachial arteriogram demonstrated an avascular cerebellar mass (Fig. 1).

Suboccipital craniectomy was performed. The bone overlying the left side of the posterior fossa was very thin. A large amount of dark-greenish fluid was evacuated from the left cerebellum. A small solid mass, which was thought to be the mural nodule of a cystic tumor, was removed from the wall of the cavity.

Pathologic analysis of the nodules revealed a vascular malformation (Fig. 2).

Postoperative course was uneventful.

Case 5. A 26-year-old man was brought to the hospital in deep coma. Several weeks prior to hospitalization he developed headaches and difficulty walking. However, these complaints were mild and he did not seek medical attention. Shortly before admission he had severe headaches and rapidly became unresponsive.

On examination the patient was deeply comatose. There was Cheyne-Stokes respiration. Oculocephalic and corneal reflexes were absent. There was no response to painful stimulation. All extremities were flaccid. The deep tendon reflexes could not be elicited.

Lumbar puncture revealed grossly bloody cerebrospinal fluid with elevated pressure.

Skull and chest x-rays were negative.

The patient expired a few hours after admission.

At autopsy there was a large fresh cerebellar hemorrhage which had ruptured into the fourth ventricle. No arteriovenous malformation was found.

Case 6. A ten-week, postpartum 24-year-old woman entered the hospital because of headaches, vomiting, and trouble with balance. Since the birth of her child she had difficulty walking because of a tendency to fall toward the right side. Several weeks later she began to

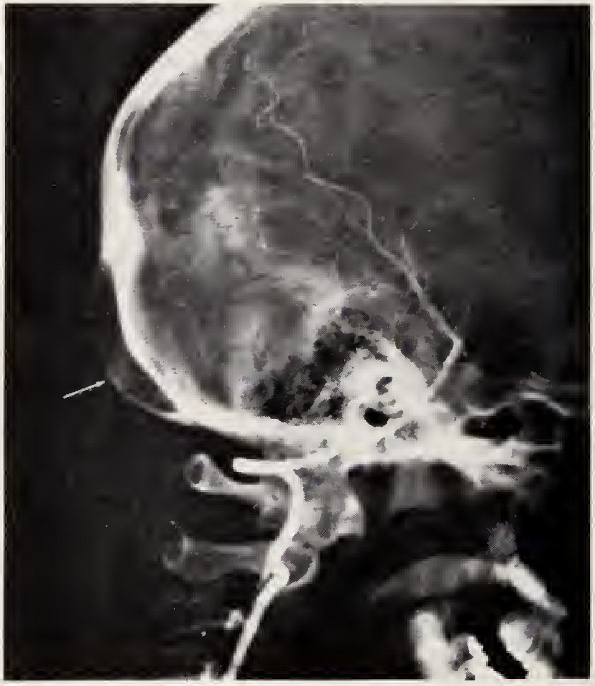

FIG. 1. Case 4. Left brachial angiogram demonstrating "tight" posterior fossa. Ballooning and thinning of bone overlying left side of posterior fossa (arrow).

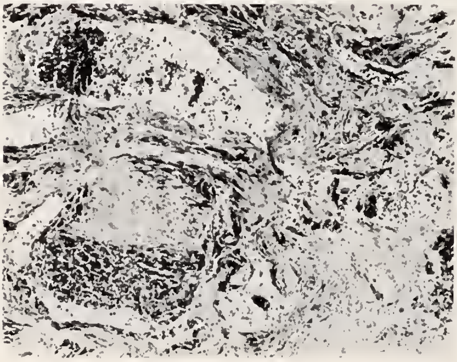

FIG. 2. Case 4. Abnormal vessels of malformation found in "mural nodule."

have intermittent episodes of headache and vomiting. These symptoms slowly increased in frequency and severity. Shortly before admission her right hand became clumsy and weak.

General physical examination was normal.

On neurologic examination there was hypotenia, intention tremor, and poor rapid alternating movements of the right extremities. Abnormal posturing of the right hand was present. The patient walked with an ataxic gait and tended to fall toward the right. The rest of the neurologic examination was normal.

Skull and chest x-rays were normal. Pneumoencephalogram demonstrated mild hydrocephalus with a shift of the vallecula toward the left. Right brachial arteriogram revealed a paramedian cerebellar mass (Fig. 3).

Suboccipital craniectomy was performed. A hematoma was encountered in the paramedial region of the right cerebellum. A small tangle of abnormal vessels in the hematoma wall was excised.

Pathologic examination confirmed the diagnosis of arteriovenous malformation (Fig. 4).

The patient made a complete neurologic recovery.

Case 7. A 16-year-old boy was brought to the hospital in coma. He was well until shortly before admission when he suddenly developed severe headaches and rapidly became comatose.

General physical examination was normal.

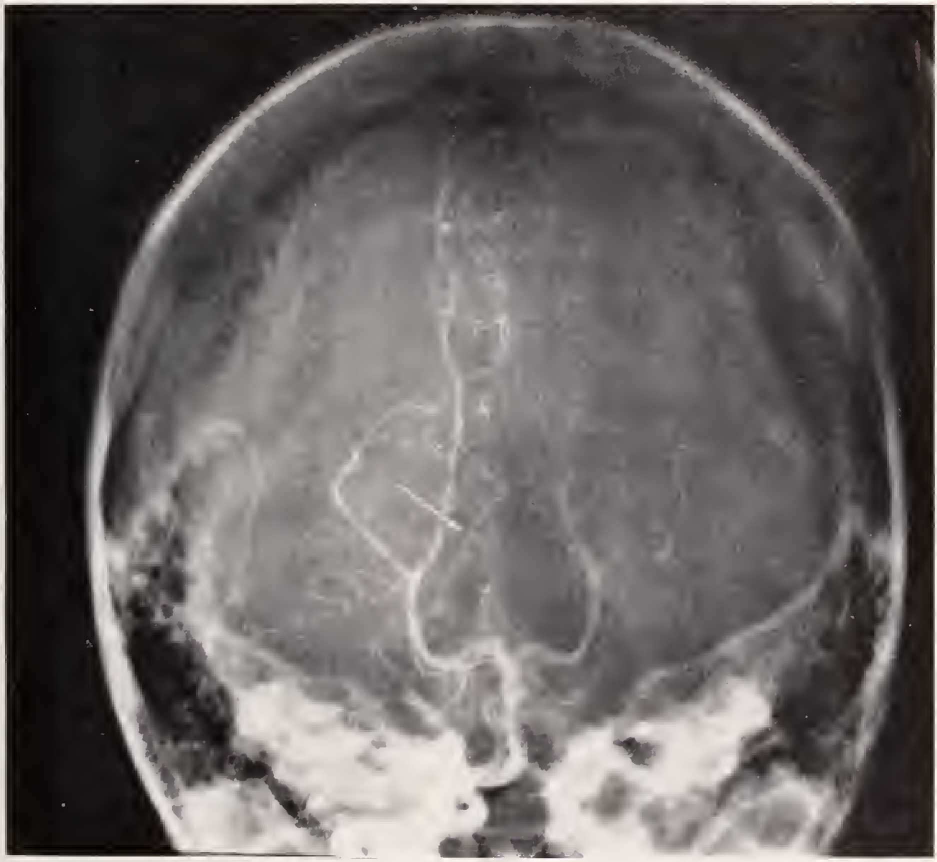

Fig. 3. Case 6. Right brachial angiogram demonstrating shift of superior cerebellar artery (arrow) toward left.

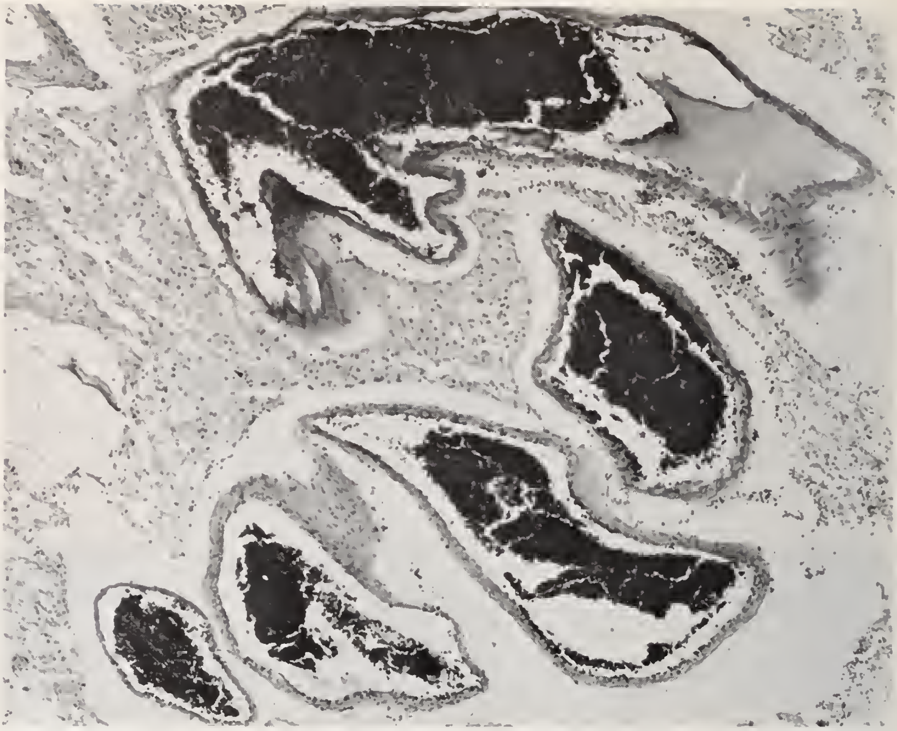

FIG. 4. Case 6. Abnormal vessels of malformation.

On neurologic examination there was minimal response to painful stimulation. The retinal veins were engorged. Corneal reflexes were depressed. Nystagmus was present on left lateral gaze. There was weakness of the right extremities. Bilateral Babinskis were present.

Cerebrospinal fluid was grossly bloody.

Skull and chest x-rays were negative. Bilateral brachial angiography demonstrated a left cerebellar mass (Fig. 5). Early drainage into the left petrosal vein was seen.

Suboccipital craniectomy was performed. A large left cerebellar hematoma was evacuated. In the wall of the hematoma cavity was a tangle of abnormal vessels, which were coagulated and excised.

Pathologic examination confirmed the diagnosis of vascular malformation.

Postoperative recovery was uneventful.

Analysis of Cases

The ages of the patients ranged from four to 35 years, with an average age of 18 years. There were five females and two males. Symptoms were present for hours to months before they became severe enough to require hospitalization. The onset of symptoms was sudden and catastrophic in three patients. In Case 3, this occurred 11 months after an episode of cerebellar hemorrhage from which the patient spontaneously recovered. Case 5 had relatively mild symptoms for several weeks, then rapidly became comatose. Four patients exhibited a rather chronic progressive course with a clinical picture essentially indistinguishable from a cerebellar tumor. In this regard, Case 4 is of interest. A history of

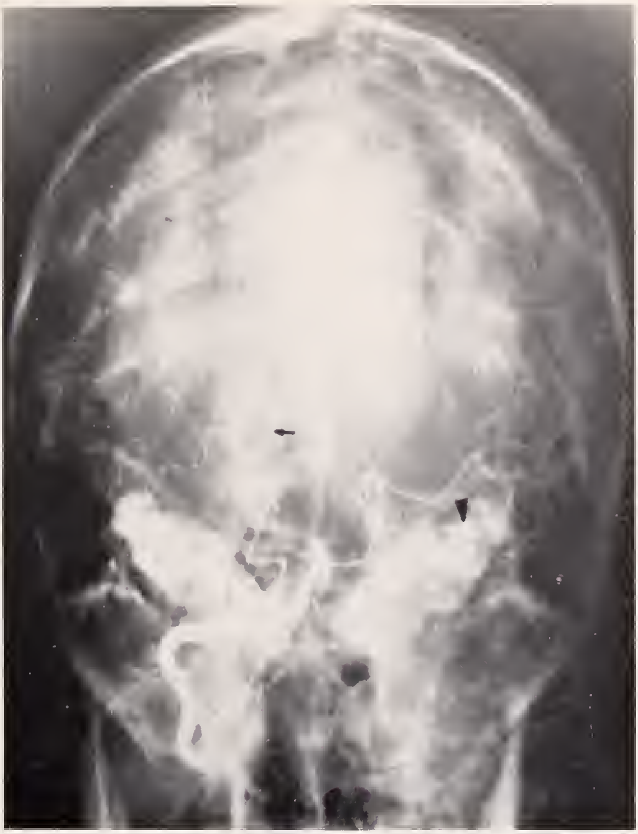

Fig. 5. Case 7. Bilateral brachial arteriogram demonstrating marked shift of posterior inferior cerebellar artery to right (arrow). Early venous drainage to left petrosal vein complex noted (arrowhead).

symptoms for five weeks was elicited. However, the thinning and ballooning-out of bone overlying the left side of the posterior fossa suggest that the hematoma was present in a mild chronic form for a much longer time.

The initial hemorrhage in Case 3 followed a mild head injury. Case 6 developed symptoms of cerebellar dysfunction shortly after the birth of her child.

Preliminary laboratory studies carried out prior to neuroradiologic diagnostic tests were relatively unrevealing. Spinal fluid was grossly bloody in the three instances where the onset of symptoms was acute. In the more chronic cases, cerebrospinal fluid findings were unremarkable.

Plain skull x-rays were negative except for the enlarged posterior fossa mentioned above. Brachial angiography was of value in localizing the hematoma but did not visualize the malformation. Early venous drainage was seen in one case.

In five of the seven cases a small arteriovenous malformation proved to be the cause of the hematoma. A malformation was recognized at surgery in two instances. In Case 4 the malformation was thought to be a mural nodule within a cystic tumor. The correct diagnosis was made after microscopic examination. In Case 2 routine biopsy of the hematoma wall revealed abnormal vessels of the malformation.

The clinical features are summarized in Table I.

Discussion

Posterior fossa vascular malformations have long been recognized as an important source of cerebellar hemorrhage. Most reports in the literature deal with large malformations. In 1939 Müller (16) described a patient with cerebellar hemorrhage caused by rupture of a small arteriovenous malformation. Odom et al (14), Crawford and Russell (9), and others (4, 6, 10, 17–23) also reported cases of cerebellar hematomas secondary to small malformations. In 1966 McCormick and Nofzinger (12) found a total of 44 "cryptic" cerebellar malformations in the literature. Subsequently McCormick et al (15) described 52 cases of cerebellar arteriovenous malformations. The majority measured less than 2 cm and could be considered "cryptic." Thus it appears that these small cerebellar vascular malformations are not as rare as previously thought.

McCormick and Nofzinger (12) pointed out that in contrast to large arteriovenous anomalies, which are mainly supratentorial in location, "cryptic" malformations are found almost equally in supra- and infratentorial locations. Their report included pathologic material consisting of lesions which had not bled. However, there seems to be a supratentorial predominance when considering cryptic malformations which have bled and produced hematomas (9, 10, 18).

The actual percentage of spontaneous cerebellar hemorrhage proven to be secondary to "cryptic" arteriovenous malformations varies considerably in the literature. Five of seven patients in the present series had such lesions. McCormick and Nofzinger (12) state that 24% of 21 cases of spontaneous cerebellar hemorrhage were caused by vascular malformations. In contrast, only one of 32 cases of nontraumatic cerebellar hemorrhage reported by Hyland and Levy (4) had a small malformation. Other authors (1, 3, 7, 8, 24–28) found no malformations.

Whether secondary to "cryptic" vascular malformations or of unknown etiology, spontaneous cerebellar hematomas tend to occur in a younger age group compared to hypertensive patients (4, 5, 9, 14, 19, 29). This finding is similar to cases of supratentorial spontaneous intracerebral hemorrhage (11, 13, 20, 30–32).

The clinical course is variable. The patient may have an acute subarachnoid hemorrhage without localizing signs. Coma may rapidly be followed by death before diagnostic tests and definitive therapy can be instituted. Pupillary abnormalities, disorders of conjugate gaze, and respiratory changes have been stressed by some authors (1, 4, 5, 8, 33). A subacute course may occur with nonspecific complaints such as headaches, vomiting, and dizziness. Some cases have a more chronic course indistinguishable from a cerebellar tumor. Cerebellar

TABLE 1

Case	Age, sex	Signs and symptoms	Duration of symptoms before admission to hospital	Roentgenologic findings	Operative findings	Pathology	Result
1	35 F	Headaches, vomiting, ataxia, papilledema	5 months	PEG—Anterior displacement of 4th ventricle	Hematoma of vermis	Hematoma wall	Recovered
2	4 F	Headaches, vomiting, papilledema, nystagmus, ataxia	3 weeks	PEG—Shift of 4th ventricle to left	Right cerebellar hematoma	Vascular malformation	Recovered
3	9 F	Headaches, vomiting, papilledema, deep coma	Several hours. Spontaneous recovery from cerebellar hemorrhage 11 months before			Cerebellar hematoma with rupture into 4th ventricle. Vascular malformation	Expired
4	12 F	Headaches, vomiting, ataxia, head tilt	5 weeks	Ballooning left posterior fossa. PEG—Non-filling of ventricles. Brachial angiogram—left cerebellar mass	Cyst with "mural nodule"	Vascular malformation	Recovered
5	26 M	Headaches, ataxia, then sudden coma, Cheyne-Stokes respiration	2 weeks			Cerebellar hematoma with rupture into 4th ventricle	Expired
6	24 F	Headaches, vomiting, ataxia	10 weeks (postpartum)	PEG—Shift of vallecula toward left. Brachial angiogram—Right paramedian mass	Paramedian cerebellar hematoma with abnormal tangle of vessels in wall	Vascular malformation	Recovered
7	16 M	Headaches, nystagmus, hemiparesis, semicoma	Hours	Brachial angiogram—cerebellar mass with early venous drainage	Cerebellar hematoma with abnormal tangle of vessels in wall	Vascular malformation	Recovered

infarction (22, 34) at times may be difficult to differentiate clinically from cerebellar hemorrhage. Bleeding may recur years after the patient survived an initial cerebellar hemorrhage (23).

Brachial or vertebral angiography is of value in localizing the hematoma (35). In contrast to large lesions where there is persistent staining and changes in cerebral circulation, considerable difficulty may be encountered in visualizing a small malformation. The feeding artery of a "cryptic" malformation is more likely to be a branch of a normal-sized major vessel. At times only a single large shunting vein to a major venous channel is seen (36). Air or air-pantopaque contrast studies help further to localize the mass.

The acute onset of cerebellar hemorrhage is associated with a high mortality rate (5, 17, 22, 29). Rapid death frequently occurs before definitive treatment is undertaken. However, surgical results are excellent in subacute or chronic cases of cerebellar hematoma. Many cases have been reported in which the hematoma has been successfully evacuated and the malformation, if present, coagulated or excised (2, 4, 6, 9, 14, 20, 24–28, 37–48). All five patients in the present report did well following surgery. McKissock et al (5) collected the largest operative series of non-traumatic cerebellar hemorrhage. Suboccipital craniectomy was performed on 14 of their patients with an operative mortality rate of 36%. These authors point out the important influence of the patient's preoperative level of consciousness on the mortality rate. No death occurred in their five patients who were alert prior to surgery.

The problems related to removal of large posterior fossa malformations are well known. In contrast, "cryptic" lesions are usually easily excised or coagulated. At times the abnormal tangle of vessels is readily identified in the hematoma wall during surgery. The malformation may resemble a mural nodule within a cystic tumor and its true nature not be recognized until microscopic examination has been carried out. Even if no suspicious area is seen, routine biopsy of the hematoma wall may reveal the correct diagnosis. When no malformation is discovered during surgery or at autopsy, the possibility exists that the abnormal vessels were destroyed at the time of hematoma formation (9, 10, 13, 14, 30, 31, 49).

Summary

1. "Spontaneous" cerebellar hematomas are uncommon.
2. In some cases a small "cryptic" vascular malformation is the cause of the cerebellar hemorrhage.
3. The clinical course may be acute, with death occurring rapidly. Differentiation from subarachnoid hemorrhage secondary to other causes may be difficult.
4. Subacute and chronic cases mimic cerebellar tumors. Surgical results are excellent in these patients.
5. Biopsy of the hematoma wall may yield an arteriovenous malformation even though no suspicious area is seen.

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Unusual Presentations of Tuberculous Meningitis

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Introduction

Tuberculosis is a subject still receiving much attention in the medical literature. Recent reviews have discussed the clinical syndromes of tuberculous peritonitis (1), pericarditis (2), osteomyelitis and arthritis (3), but few reports of tuberculous meningitis have appeared in the current literature. O'Toole et al (4) focused attention on steroid therapy and did not discuss the protean manifestations of this disease. A more recent paper from Nigeria (5) reported on cases, mainly in children, in whom malnutrition may have affected the clinical presentation. Kocen et al (6) did discuss some unusual manifestations of tuberculous meningitis. In the reports cited above, most patients were either indigent, debilitated, or malnourished. We describe unusual presentations of tuberculous meningitis in three well-nourished women encountered during a nine-month period.

Case Reports

Case 1. A 32-year-old black, female nurse was admitted because of 3 months of headaches and fever. Two years previously her left knee became swollen. She was seen by her physician who found no evidence of systemic disease and the swelling subsided. One month before admission her left knee became swollen again; her physician prescribed phenylbutazone which relieved the symptoms. Excruciating global headaches ensued with night sweats and a 5-pound weight loss. She received 10 days of tetracycline and then 7 days of erythromycin prior to admission to The Mount Sinai Hospital. Two days before admission her temperature rose to 103°F. Physical examination on admission revealed a well-nourished female with a regular pulse of 88 beats/minute; rectal temperature of 102.6°F; blood pressure of 100/60 mm Hg. Positive physical findings were resistance to neck flexion and bilateral, nontender effusions of her knees. Laboratory data revealed a hemoglobin of 11.7 g/100 ml; white blood cell count of 5,200/mm^3 with 4 band forms, 64 mature neutrophiles, 30 lymphocytes, and 2 monocytes; erythrocyte sedimentation rate of 85 mm/hr (Westergren). Urinalysis showed a trace of protein and 7 white blood cells and 3 red blood cells per high power field.

The platelet count was 605,000/mm^3; serum electrolytes, blood urea nitrogen, and serum creatinine were normal. The serum glutamic oxaloacetic transaminase was 45 international microunits (IuU); serum glutamic pyruvate transaminase was 38 IuU; alkaline phosphatase was 100 IuU; bilirubin was 0.7 mg/100 ml; glucose was 90 mg/100 ml; total protein was 7.2 g/100 ml; serum phosphorus was 3.6 mg/100 ml. Latex fixation test and lupus erythematosus cell preparations were negative. Serum protein electrophoresis was normal. Roentgenograms of the cervical spine, cranium, sinuses, and chest were negative, as was the the brain scan. Soft tissue swelling around the knees but no joint destruction was evident on the roentgenograms. Lumbar punctures were performed and the knee effusions were aspirated (Table I). Lumbar punctures persistently showed a predominance of polymorphonuclear

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TABLE I

Clinical Data

Date	Appearance	Opening Pressure (cm H_2O)	Red Blood Cells (per mm³)	White Blood Cells (per mm³)	Neutrophiles (per mm³)	Lymphocytes (per mm³)	Glucose (mg/100 ml)	Protein (mg/100 ml)	Acid Fast	
									Stain	Culture
A. Spinal Fluids										
Case 1										
5/10	clear	5	8	96	83	13	32	25	Neg	Neg
5/11	clear	7	6	28	10	8	32	25	Neg	Neg
5/13	clear	14	2	71	52	19	48	43	Neg	Neg
5/14	clear	—	8	96	83	13	38	52	Neg	Neg
5/15	turbid	—	0	188	172	16	28	34	Pos	Pos
5/17		19	9	101	86	15	32	82	Pos	Pos
5/18		—	38	242	223	19	26	66		—
5/20			20	99	74	25	—	—		Pos
5/26			0	215	170	45	30	112		Pos
6/2			6	34	30	4	40	116		
6/9			0	34	6	28	30	86		
6/16			2	29	15	14	34	107		
6/23			64	82	14	68	32	110		
Case 2										
5/24	clear	25	0	39	4	35	22	39	Neg	Pos
5/30	clear	18	6	30	4	26	26	33	Neg	
6/2	clear	11	0	83	3	80	62	18	Neg	
6/9	clear	9.5	0	127	21	106	130	94	Neg	
6/10	clear	—	0	5	2	3	130	47	Neg	
Case 3										
8/9	clear	40	0	200	17	183	42	46	Neg	Pos
8/12	clear	60	0	306	56	250	46	66	Neg	

B. Joint Fluid

Date	Appearance	Red Blood Cells (per mm³)	White Blood Cells (per mm³)	Neutrophiles (%)	Monocytes and Lymphocytes (%)	Glucose (mg/100 ml)	Protein (mg/100 ml)	Acid Fast	
								Stain	Culture
Case 1									
5/12	turbid	3010	2900	4	96	0	4.4	Neg	Pos
6/6	turbid	1350	6100	33	67	0	3.5	Neg	

cells; the patient was begun on intravenous ampicillin 12 grams per day for the possibility of a partially treated bacterial meningitis. The first strength and intermediate purified protein derivative skin tests were negative, but the second strength purified protein derivative test was positive. Gram stains, India ink preparations and acid fast stains were performed on all spinal fluids and joint effusions. The acid fast stain of the fluid of the fifth lumbar tap was positive, and triple therapy with isoniazid (INH) 600 mg qd, ethambutol 600 mg qd and streptomycin 1 gm qd with pyridoxine was begun. Cultures of this spinal fluid and the knee aspirate yielded M. tuberculosis. The patient became afebrile 21 days later although the spinal fluid still revealed a predominance of polymorphonuclear leukocytes. It was not until 30 days after admission that lymphocytes predominated in the cerebrospinal fluid.

Case 2. A 26-year-old white married woman was admitted with headache and left hemiparesis. Four months before admission this primiparous patient was 33 weeks pregnant when parietal headaches, photophobia, and anorexia began insidiously. Two weeks later she was admitted to a hospital with increasing confusion and disorientation. Delivery ensued suspected and heparin and corticosteroids were administered. During this period the and heavy postpartum hemorrhage occurred. Disseminated intravascular coagulation was patient had fever and night sweats. Lumbar puncture showed a lymphocytosis and decreased glucose concentration. The patient was given isoniazid and para-aminosalicylic acid which she received until discharge 3 weeks later when her physician discontinued the medication. Two weeks later, the medication was reinstituted when the spinal fluid culture grew acid fast organisms. However, since the niacin test was negative and a rash developed, the drugs were discontinued again.

She remained well until 6 days prior to admission when right-sided weakness and paresthesias developed followed by recurrence of headaches accompanied by vomiting and blurred vision. She was unable to walk when admitted to The Mount Sinai Hospital. The history of organisms in the spinal fluid and anti-tuberculous medications was not known by the attending physicians at Mount Sinai.

Physical examination on admission revealed a rectal temperature of 99°F; a regular pulse of 80 beats/minute; blood pressure of 120/85 mm Hg; respiration of 14/minute. The neck was stiff to flexion and neurologic examination revealed a confused, disoriented patient with a right-sided hemiparesis. Laboratory data included a hemoglobin of 15.1 g/100 ml; white blood cell count of 4,900/mm^3 with 75 mature neutrophiles, 9 band forms, 14 lymphocytes, and 2 monocytes; erythrocyte sedimentation rate of 20 mm/hr; platelet count of 272,000/mm^3. The veneral disease research laboratory test, lupus erythematosus cell preparations, and urinalysis were negative. Serum electrolytes and the sequential multichannel analyzer 12 were normal. Roentgenograms of the chest and brain scan were negative. Lumbar puncture was performed and both stains and cultures for bacteria, fungi, and acid fast organisms were negative (Table I). The electroencephalogram (EEG) showed bilateral cerebral dysfunction, and cerebral angiography revealed dilated ventricles. Intermediate and second-strength purified protein derivative, tests were negative on admission but a repeat second strength skin test was positive on the 26th hospital day. The patient was initially given intravenous dexamethasone 4 mg q6h for increased intracranial pressure and isoniazid 300 mg qd. Three weeks after admission, the initial spinal fluid culture grew M. tuberculosis. Ethambutol 1.2 gm qd, streptomycin 500 mg bid, and pyridoxine were added. All neurologic symptoms and signs quickly disappeared, and the spinal fluid glucose concentration rose to normal by the 8th day of isoniazid therapy.

Case 3. A 27-year-old, married, white woman was admitted with headache, fever, lethargy, and decreasing mental function. This patient was well until 7 days prior to admission when frontal headaches began while she was visiting in San Francisco. The patient felt warm but the temperature was not obtained. The following day in Los Angeles, the patient was given erythromycin in a dose of 250 mg every 6 hours which she took for 4 days until arriving in Las Vegas where another physician prescribed barbiturates for persistent headaches. One day prior to admission she developed chills, fever, restlessness, irritability, and confusion. She was noted to have a stiff neck and hospitalization was advised. Physical examination on admission revealed a confused, lethargic, well-developed and well-nourished female. The rectal temperature was 103°F; blood pressure was 120/80 mm Hg; pulse was 68/minute; respiration was 14/minute. Nuchal rigidity was observed, although the deep tendon reflexes were equal and brisk bilaterally and the plantar reflexes were flexor.

Laboratory data on admission revealed hematocrit of 44%; white blood cell count of 5,000/mm^3 with 9 band forms, 62 mature neutrophiles, 22 lymphocytes, and 7 monocytes; erythrocyte sedimentation rate was 18 mm/hr. The urinalysis was negative. Other laboratory data included total serum protein 6.4 g/100 ml with 3.9 g/100 ml of albumin. Negative tests for Salmonella and Brucella antibodies and heterophile agglutinins were obtained. Throat, stool, urine, and blood cultures were also negative. Spinal puncture was performed (Table I) and smears for bacteria, fungi, and acid fast organisms were negative. Roentgenograms of the chest and skull and an echoencephalogram were negative. An intermediate strength purified protein derivative test was negative on admission. The patient's clinical course was one of rapid deterioration with increased intracranial pressure developing and bilateral Babinski reflexes noted the second hospital day. Dexamethasone 4 mg q6h was administered, but by the third hospital day the patient lapsed into deep coma with Cheyne-Stokes respirations. She was given ampicillin 12 g intravenously per day while the diagnostic workup proceeded. On the eighth hospital day isoniazid 300 mg and 1 gm of streptomycin intramuscularly were added. Fever persisted and the patient died on the twelfth hospital day. Culture of the spinal fluid 4 weeks later yielded M. tuberculosis.

Discussion

The three patients had the following clinical findings in common: they were women (ages 26–27, 32); they were previously in good health with no signs of disease in other organs (although the second patient was pregnant); they were from middle income backgrounds; none had known contact with tuberculosis, although the first patient was a private duty nurse in a general hospital; and none had pulmonary tuberculosis. The manifestations and clinical presentations of their disease were somewhat unusual and unexpected.

The first patient had a predominance of polymorphonuclear leukocytes on the initial spinal puncture; this is not unusual and may occur early in the disease (6). However, the persistence of this pattern for 30 days with concomitant clinical improvement has not been described. Although this patient had symptoms for 3 months, her spinal fluid suggested the possibility of partially treated meningitis in view of recent therapy with antimicrobial agents. Acid fast stain of the fifth spinal fluid specimen revealed 2 acid fast bacilli. This finding occurs in only 10% of cases of tuberculous meningitis (7). Tuberculous arthritis, usually monoarticular, is most common during the first 14 years of life but may occur at any age (3). Physical signs without roentgenographic evidence of joint or bone destruction may be present for months, occasionally even years. Recently tuberculous arthritis has been more commonly seen in adults (8). The joint fluid findings suggested the possibility of tuberculosis.

The second patient apparently developed her disease during pregnancy, an event which may precipitate the disease (9). Presentation with hemiparesis was also unusual (6), and in a febrile patient would make tuberculous meningitis a less likely diagnosis. The "spontaneous" improvement was unexplained until the history of having received corticosteroids and antituberculous therapy was obtained. In this patient dexamethasone therapy was administered concomitantly with isoniazid therapy. Although improvement of tuberculous meningitis with isoniazid alone may occur as described by Lepper et al (7), triple therapy with isoniazid, streptomycin and ethambutol was administered. The latter drug is known to enter the cerebrospinal fluid (10) and has been used with efficacy in this disease. However, we are unaware of any prospective studies comparing isoniazid and ethambutol with isoniazid, pyridoxine and streptomycin in the treatment of tuberculous meningitis.

The mortality of tuberculous meningitis has decreased from 85% to 31–33% with the advent of isoniazid therapy (7, 8). Mortality has been associated with increasing age (9, 11), miliary tuberculosis (5), pulmonary tuberculosis (9), underlying disease (5), malnutrition (5), and the level of consciousness at the time of admission (7, 9). The third patient was previously in good health but had a fulminant clinical course with only a 20 day interval from the initial headache to her demise. The clinical presentation with lymphocytosis in the cerebrospinal fluid, with a normal cerebrospinal fluid glucose and protein concentration as well as normal roentgenogram of the chest, hemogram, erythrocyte sedimentation rate, and negative purified protein derivative skin test may be seen in tuberculous meningitis (6), but make the early diagnosis of this entity very difficult. Rapid clinical deterioration with progressive loss of consciousness is often prognostic of a fatal outcome. Although corticosteroids, isoniazid, and streptomycin were administered, the patient failed to respond.

Summary

Three cases of tuberculous meningitis with unusual presentation occurring in previously healthy females are presented. It is suggested that this diagnosis be considered in every patient with unexplained neurologic manifestations and meningitis, whether polymorphonuclear cells predominate in the cerebrospinal fluid or the glucose and protein content are normal. Therapy with at least isoniazid should be started while the diagnostic investigation is pursued.

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Pulmonary Lymphatics in Relation to Pulmonary Clearance and Interstitial Fluid and Their Possible Implication in the Pathogenesis of Emphysema

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Physiologic studies have amply demonstrated the importance of the pulmonary lymphatics in alveolar clearance of fluid and particulate material and in the maintenance of proper interstitial fluid relationships. Most studies along these lines have been conducted in other species, and a dearth of knowledge still exists concerning the anatomy and functional relationships of pulmonary lymphatics in man and the degree to which they participate in the evolution of disease. Undoubtedly, the chief barrier to a proper understanding is the difficulty in adapting conventional histopathologic techniques to meet the needs of demonstrating the lymphatics in a form which approximates their condition in the living patient.

To investigate the deep set of pulmonary lymphatics, which are probably of chief concern in alveolar clearance, I used a technique I devised in 1935 (1) through which the lymphatics are perfused and fixed in maximal distention by means of a continuous slow intrabronchial drip of 10 percent formalin solution over many hours, while inflation of the lung is maintained in a negative pressure chamber (Figs. 1, 2) within the physiologic range (minus 10 to 20 centimeters of water).

Fixation is generally complete after perfusion with 2 to 3 liters of formalin, whereupon negative pressure is continued until the lungs dry. Lungs prepared in this way are suitable for routine histologic procedures and for Gough-Wentworth macrosections. Lymphatic channels in the pleura are often prominent and readily injected with dyes or air through a fine needle (Fig. 3). Intrapulmonic lymphatics are easily visualized under low magnification (Fig. 4) so that estimates of their patency obtained by direct inspection provide an adequate basis for comparative studies.

The underlying principle in fixation with this method, as suggested by numerous direct and indirect evidences, is the creation within the lung of a continuous pressure gradient sufficient to drive fixing fluid from the finer airways into the interstitial tissues, lymphatic channels, and lymph nodes (Fig. 5), whence fluid emerges ultimately at the hilus after having, in effect, lavaged the entire substance of the organ. The slow drip of formalin, by slowing the rate of fixation, gives ample opportunity for uniform perfusion of all parts. Within

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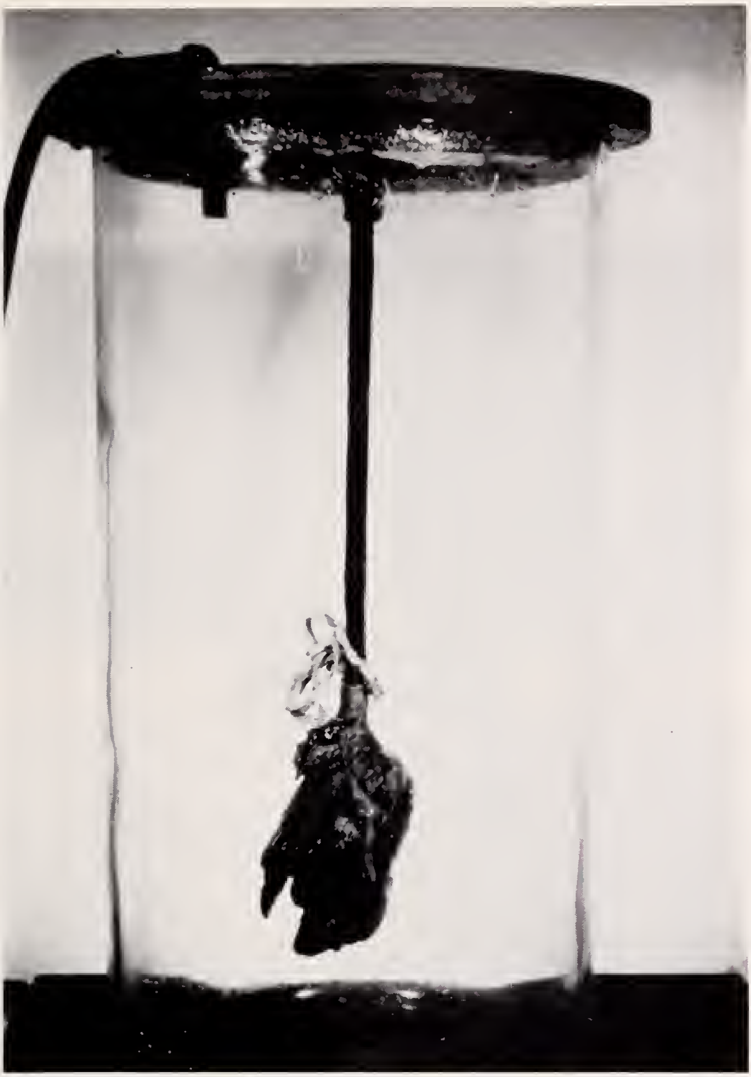

FIG. 1. Negative pressure chamber: Uninflated lung secured by main bronchus to air-feed metal tube (atmospheric pressure). Lid in place with airtight vaseline seal.

the range of pressure used (minus 10 to 20 centimeters of water), the suction gradient is generally sufficient to drive edema fluid out of the alveoli into the lymphatics. In many instances, the transport of protein-rich edema fluid within the interstitial fabric enroute to lymphatics leaves its mark in the form of a homogeneous deposit of inspissated proteinaceous material which seems to blend with connective tissue fibers, in striking mimicry of hyalin or amyloid (Figs. 6 and 7) unless differentiated by special stain. No instance has been encountered of air within the lymphatic channels or interstitial tissues ("interstitial emphysema").

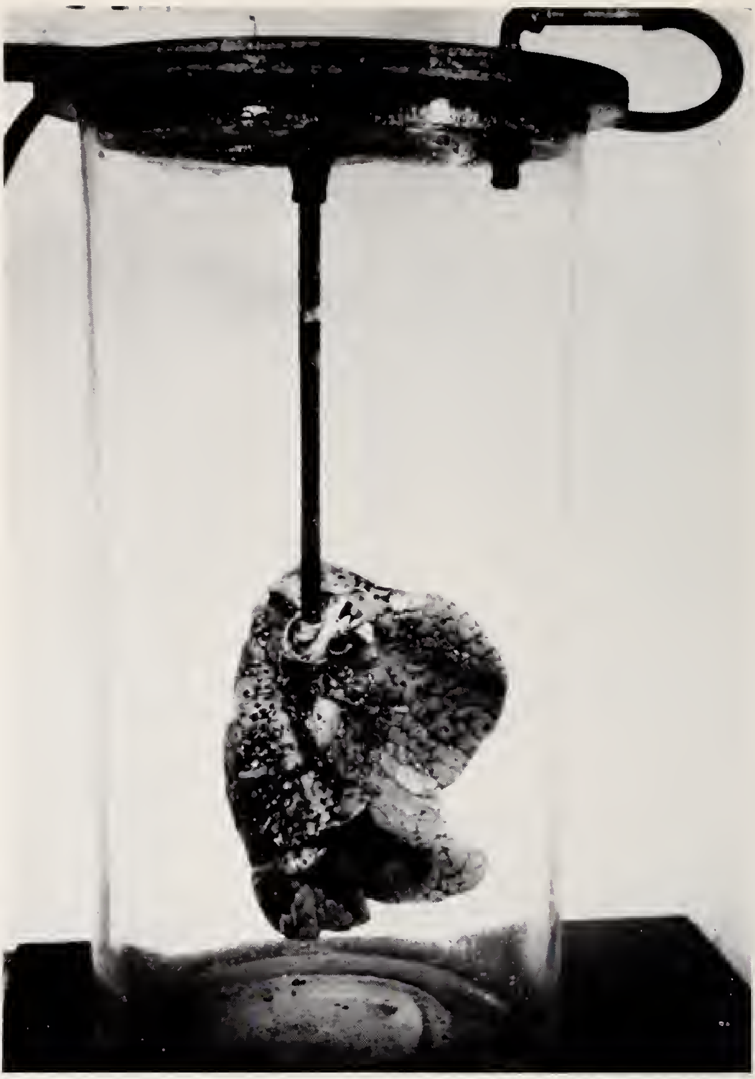

Fig. 2. Negative pressure chamber: Lung completely fixed in inflated state.

A distinctive artifact inherent in the method is its production of a sort of artificial interstitial edema. The edema-like swelling of the supporting framework is especially prominent where the interstitium has been the seat of acute or chronic inflammation attended with edema and newly laid down fibrous tissue. In many of these sites the lymphatic channels are also unusually wide and numerous (Fig. 8) so that, taken as a whole, the histologic pattern can be taken to represent chronic lymphedema of pulmonary tissue, differing only in details from chronic postmastectomy lymphedema of the upper extremity or elephantiasis of the lower extremity caused by lymphatic blockage in the upper thigh or groin.

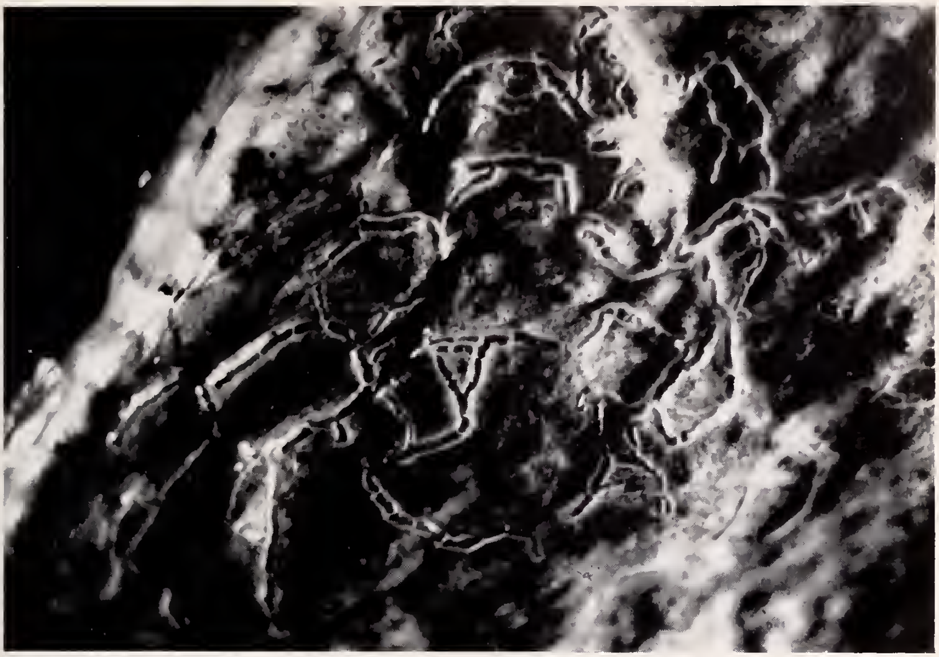

FIG. 3. Low power view of pleural lymphatics of inflated lung (injected with air).

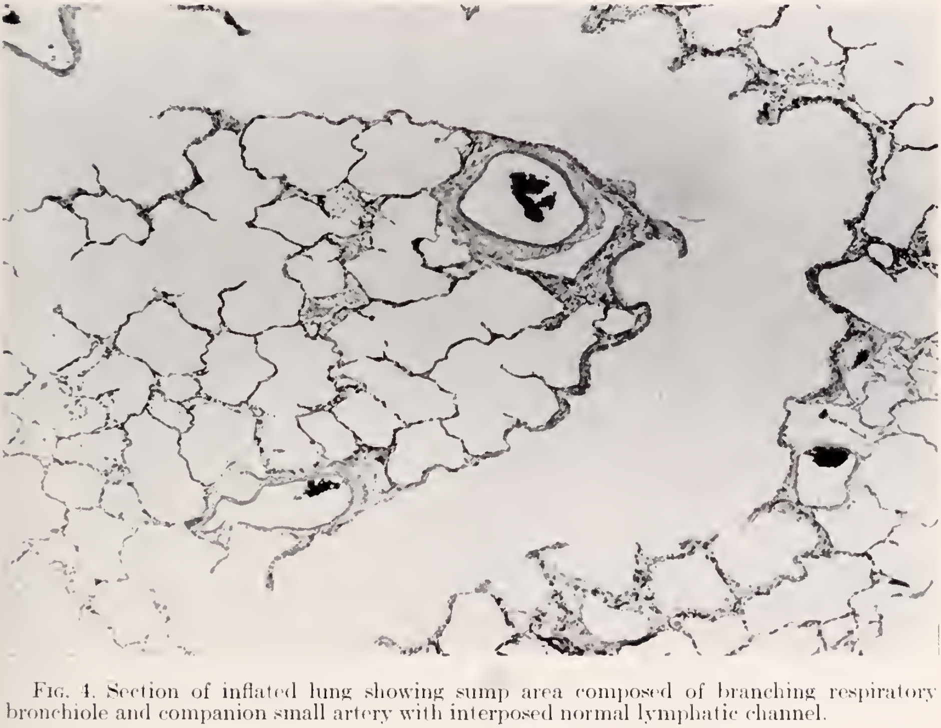

FIG. 4. Section of inflated lung showing sump area composed of branching respiratory bronchiole and companion small artery with interposed normal lymphatic channel.

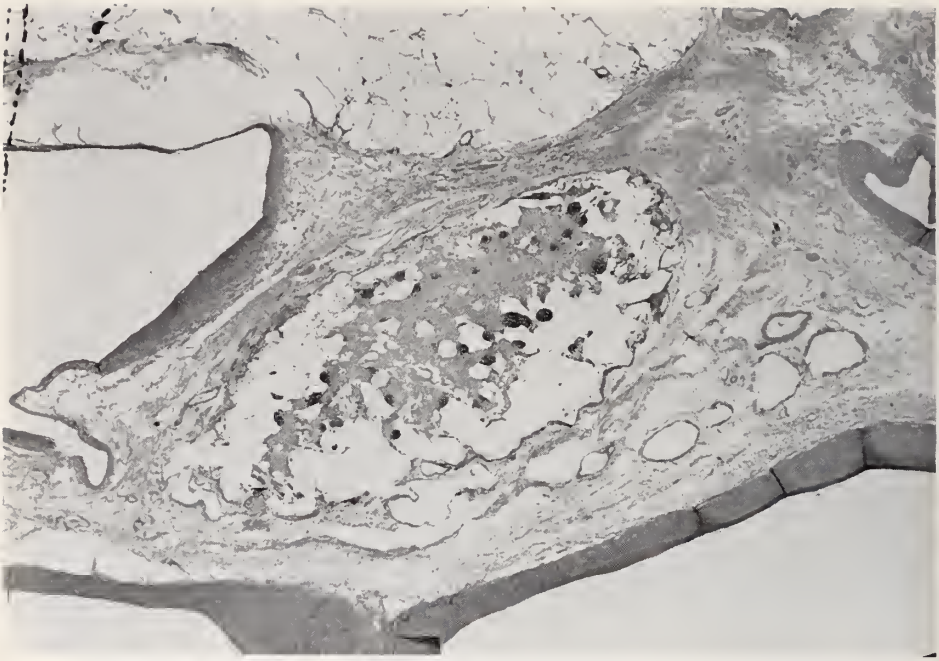

FIG. 5. "Lavaged" intrapulmonary lymph node showing distended sinuses.

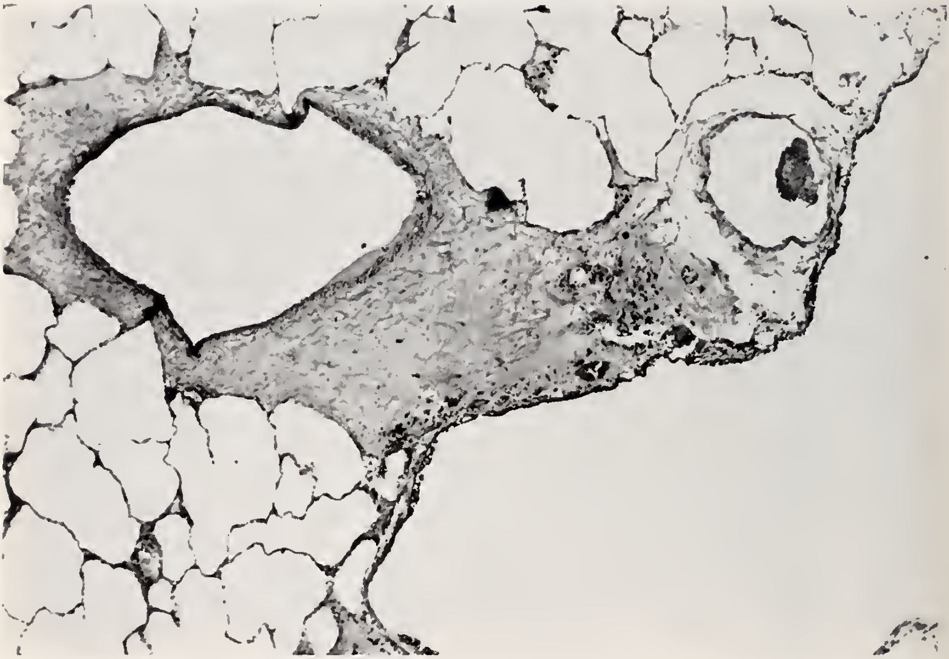

FIG. 6. Pseudo-hyalinization of connective tissue sheath of vein by proteinaceous material driven from alveoli by perfusion with slow intrabronchial drip of fixing solution while lung was kept inflated by negative pressure.

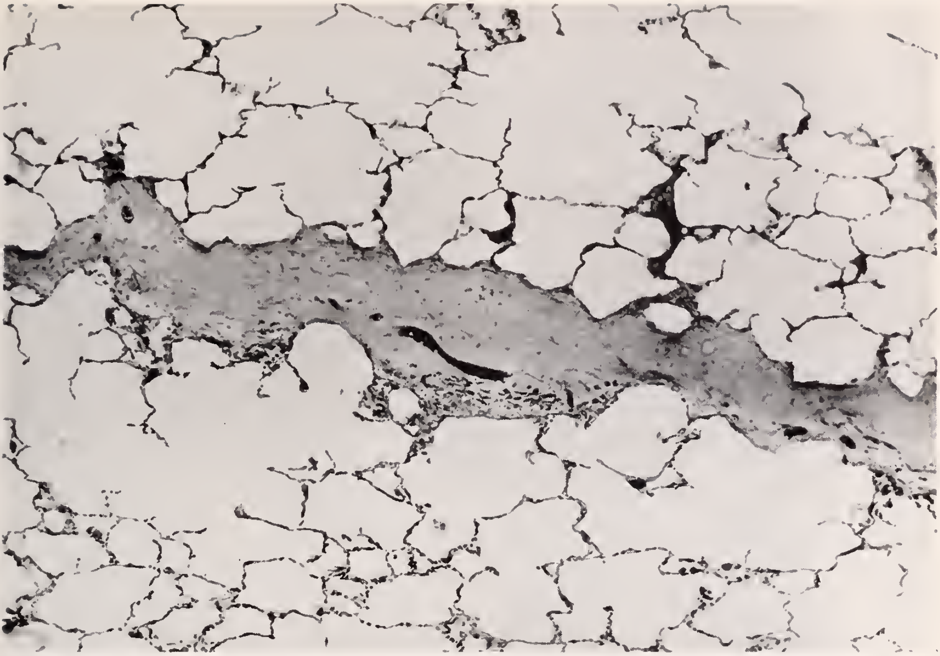

Fig. 7. Pseudo-hyalinization of interlobular septum (same lung as in Figure 6).

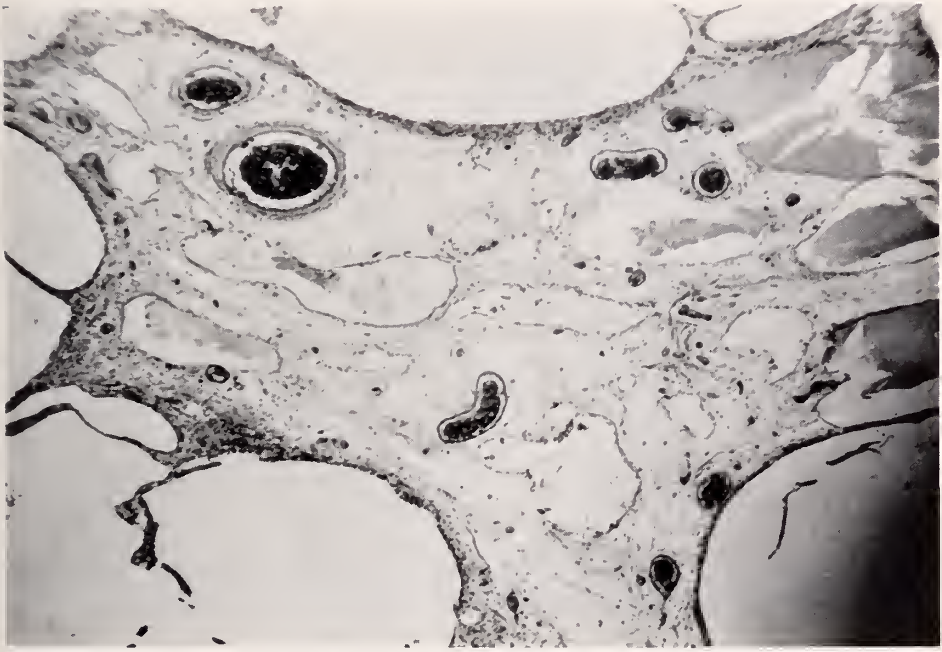

Fig. 8. Chronic lymphedema of lung (lung fixed in inflation).

The edema-like swelling of the interstitial tissues induced in this manner lays bare many details of structure of the supporting interstitium of terminal and respiratory bronchioles whereby it becomes possible to define clearly the interrelations of pigment deposits, inflammatory exudates, and fibrosis with one another and with the lymphatic vessels. In this respect the method seems better suited for the routine investigation of these elements than the more common method of distending the lung by intrabronchial injection of fixing solution under pressure.

Data reported here are drawn from a general study of pulmonary clearance mechanisms with particular reference to lymphatics. The periarterial lymphatics associated with respiratory bronchioles received particular attention as conforming most closely to the specifications of a "standard" lymph vessel of the pulmonary parenchyma and as most representative of the "rootlets" of the deep pulmonary lymphatic system capable of siphoning off fluid from the principal "sump" areas. (This is Macklin's term for sites of selective absorption of alveolar fluid [2].)

Each case was reviewed comprehensively to give proper weight to various anatomic and functional factors brought to light clinically and at autopsy insofar as they might add to or subtract from the patency of this class of lymphocytes. Because multiple factors did, indeed, exist in the majority of cases, the information outlined in Table I refers to trends rather than clearcut correlations.

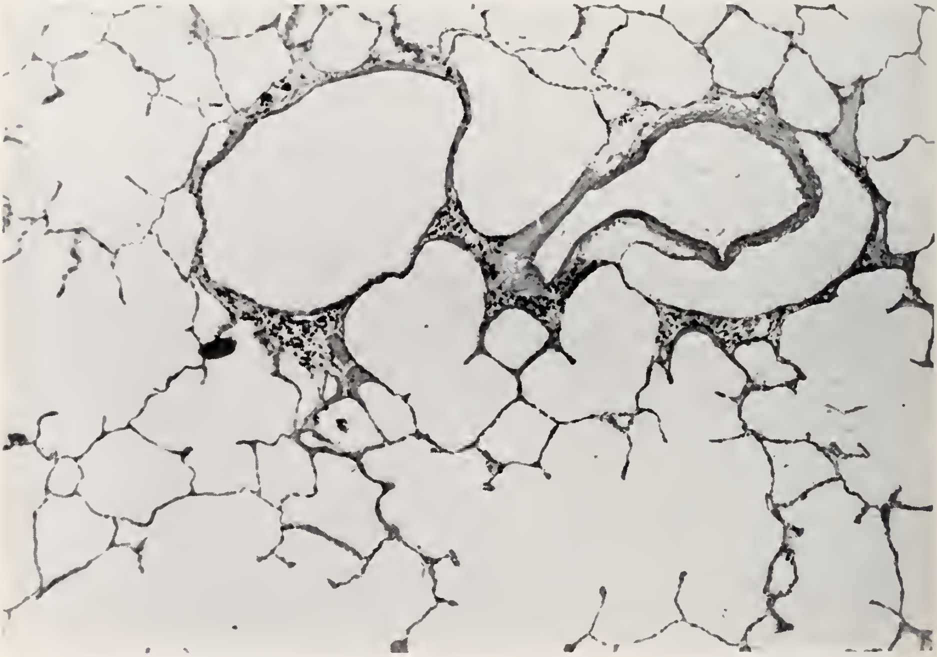

FIG. 9. Sump area showing excessively wide lymphatic (lung fixed in inflation).

The additive effect of combinations of factors on lymphatic patency is brought home impressively in connection with cases in which the pulmonary lymphatics were exceptionally wide (Fig. 9). The first seven cases listed in the following tabulation are examples of left-heart failure complicated by anasarca (overhydration), pneumonia, or both:

Conditions Associated with Exceptionally Wide Pulmonary Lymphatics

Primary disorder	Associated disorder(s)
1. Arteriosclerotic heart disease	Anasarca, bronchopneumonia
2. Arteriosclerotic heart disease	Anasarca, rheumatic heart disease (mitral insufficiency)
3. Arteriosclerotic heart disease	Anasarca, emphysema, cor pulmonale, bronchopneumonia
4. Arteriosclerotic heart disease	Myocardial infarction, acute interstitial pneumonia
5. Rheumatic heart disease (mitral stenosis)	Anasarca, chronic interstitial pneumonia
6. Rheumatic heart disease (mitral insufficiency)	Acute rheumatic myocarditis, rheumatic pneumonia, congenital interatrial septal defect
7. Rheumatic heart disease (mitral insufficiency)	Pancytopenia, septic pneumonia
8. Panlobular emphysema (cor pulmonale)	Anasarca, bronchopneumonia
9. Influenzal pneumonia	Obesity (hypervolemia)
10. Pulmonary sarcoidosis	—
11. Pulmonary histoplasmosis	—
12. Diabetic coma	Overhydration ("insulin edema")
13. Lymphatic carcinomatosis	Bronchopneumonia
14. Lymphatic carcinomatosis	Massive involvement of hilar lymph nodes
15. Lymphatic carcinomatosis	Severe bronchopneumonia (with suppuration)
16. Lymphatic carcinomatosis	Bronchopneumonia; renal shutdown; overhydration
17. Lymphatic carcinomatosis	-

Similar cases have been described previously by Heath and Hicken (3) and by myself (4) in relation to mitral stenosis, and by Uhley and his associates (5, 6) as a response to chronic pulmonary edema in dogs with experimental arteriovenous fistula and prolonged overhydration maintained by salt and desoxycorticosterone trimethylacetate.

The enhancing effect of congestion and inflammation of the lung and the contrasted effect of the shock syndrome are set forth in Tables I and II. Under the rubric 'congested lung' (7) are listed conditions associated with pulmonary venous hypertension, such as mitral valvular disease and left ventricular failure arising from coronary arterial disease. Under 'pneumonias' are listed acute and chronic forms of pulmonary inflammation of known or unknown etiology.

The shock syndrome was presumed to exist when, in addition to a suggestive clinical picture, autopsy revealed centrilobular necrosis of the liver, a bloodless spleen, and pronounced hypostatic congestion of the lung accompanied by variable edema and atelectasis ('shock lung') (8, 9). The effects of cardiogenic shock on lymphatic patency appeared to be superseded by the effects of the underlying cardiac disorder, and cases in this category are therefore included in Table I under 'congested lung.' There remained 19 cases of endotoxin shock and hypovolemic shock unassociated with congested lung or pneumonia, and in these the lymphatics were uniformly closed. A possible explanation is suggested by the histologic observation that the pulmonary arterioles and venules, being widely dilated in the shock syndrome, stretch their supporting outer sheath of connective tissue into a narrow limiting membrane, with the consequent obliteration of its contained lymphatics.

Deposits of anthracotic pigment seemed to have little effect on lymphatic patency except where the encompassing scar tissue was densely contracted and hyalinized (Fig. 10). Aging also appeared to have no independent bearing on lymphatic patency.

Because of their special position as part of the pulmonary clearance mechanism, the lymphatics were examined with particular interest in relation to the various forms of pneumonia encountered in this series (Table III). In the commonplace types caused by bacterial or viral infection and in acute or chronic

TABLE I

Patency of Primary Lymphatics of Lung in 166 Autopsies

Principal determinant	Open lymphatics	Closed lymphatics	Total
Congested lung	35	19	54
Pneumonias	27	11	38
Shock	0	19	19
Lymphatic carcinomatosis	10	3	13
Emphysema	3	9	12
Bronchitis	0	11	11
Asbestosis	0	3	3
Miscellaneous	9	7	18
Total	84	82	166

TABLE II

Pulmonary Lymphatics in Shock (58 Cases)

Lymphatic patency	Endotoxin shock		Hypovolemic shock		Total	
	Open lymphatics	Closed lymphatics	Open lymphatics	Closed lymphatics	Open lymphatics	Closed lymphatics
Shock plus congested lung	3	3	17	8	20	11
Shock plus pneumonia*	3	2	1	2	4	4
Shock with neither congested lung nor pneumonia	0	14	0	5	0	19

* Other than suppurative pneumonia or "hypostatic pneumonia."

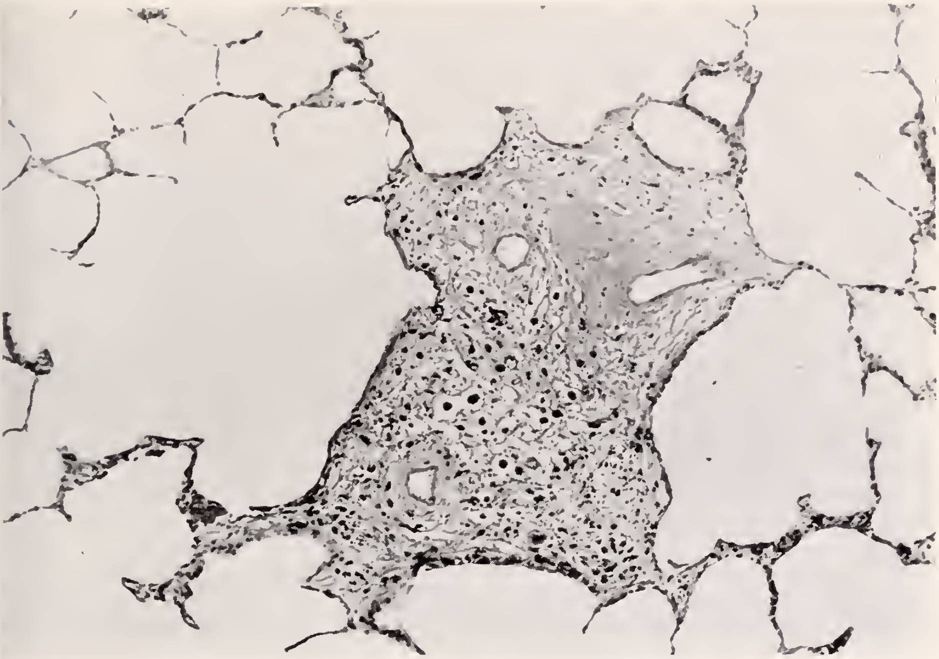

Fig. 10. Advanced anthracofibrosis of sump tissue.

TABLE III

*Primary Lymphatics in Nonsuppurative Pneumonia (31 Cases)**

Lymphatic patency	Open lymphatics	Closed lymphatics
Secondary bronchopneumonia (16 cases)	11	5
Interstitial pneumonia (10 cases)	8	2
Granulomatous pneumonia (5 cases)	5	0
Total	24	7

* Acute and chronic types unassociated with congestive heart failure or shock syndrome.

serofibrinous pneumonias of uncertain etiology centered chiefly within the interstitial tissues of the lung, the lymphatics tended to be widely patent. Chronic forms of pneumonia, particularly chronic granulomatous disorders such as sarcoidosis, histoplasmosis, and atypical mycobacteriosis, were regularly accompanied by widely open lymphatics.

The picture was quite different in the case of pneumonias associated with tissue necrosis and supervening suppuration (suppurative pneumonia) (Table IV). The role of bacterial pathogenicity seemed to be clouded in these instances by a multiplicity of predisposing elements, either systemic (alcoholism, diabetes, cachexias, and endotoxin shock) or indigenous to the lung. Except for two cases of lymphatic blockage by cancer, the principal predisposing factor in the lung consisted in localized or regional cystic disease such as centrilobular or bullous emphysema or fibrocystic disease ('honeycomb lung'). Chronic lymphedema was a prominent feature in the walls of many of the larger pus-filled saccules (Fig. 11).

Relation of Lymphatics to Emphysema

Cases of emphysema were classified as centrilobular (CLE), panlobular (PLE), or as a combination of these, as determined through study of Gough-Wentworth macrosections and histologic slides examined under low power. Mild

TABLE IV

*Primary Lymphatics in Suppurative Pneumonia (18 Cases)**

Lymphatic patency	Open lymphatics	Closed lymphatics
Centrilobular emphysema (5 cases)	2†	3
Bullous emphysema, honeycomb lung (3 cases)	3‡	0
Lymphatic obstruction (cancer) (2 cases)	1	1
Aspiration pneumonia, "spillover" pneumonia, septic pneumonia with alcoholism, cachectic pneumonia (8 cases)	3	5
Total	9	9

* Findings in uninflamed areas of lung.

† Case complicated by mitral stenosis or chronic lymphedema.

‡ Associated with chronic lymphedema.

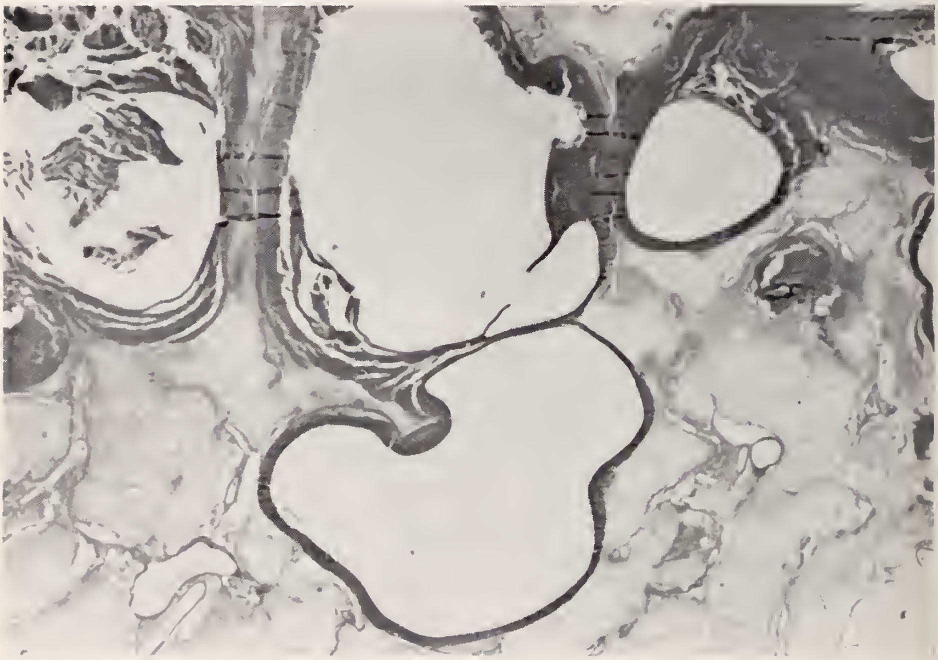

FIG. 11. Suppurative pneumonia complicating bullous emphysema associated with chronic lymphedema (lung fixed in inflation).

cases were disregarded. Of the remaining 43 cases, 25 were severe and 18 were moderate in grade. Classified by type, there were 18 cases of severe and moderate PLE (among which two exhibited advanced tissue atrophy and destruction), 11 cases of severe and moderate CLE, and 14 cases of mixed CLE and PLE.

In order to establish a baseline for evaluation of lymphatic patency, the cases were screened to eliminate coexisting conditions that might in themselves affect lymphatics, that is, pulmonary congestion, nonsuppurative pneumonias, or shock syndrome. A residual group of 21 cases was considered suitable for evaluation. Lymphatics were patent in five of the 12 cases of PLE and in none of the nine cases of CLE or of CLE combined with PLE (Table V).

Other divergences between PLE and CLE became apparent on further analysis. Table VI summarizes findings relevant to suppurative pneumonia (SP) and right ventricular hypertrophy (RVH). Suppurative pneumonia complicated nine cases of emphysema, of which seven were pure CLE or CLE mixed with PLE. The remaining two were cases of pure PLE, in one of which much cystic destruction had developed; the other was a case of moderate PLE, in which suppurative pneumonia apparently developed as a complication of endotoxin shock in the wake of B. proteus sepsis. Right ventricular hypertrophy, in which the average thickness of the ventricular wall was 1½ to 2 times normal (6–8 mm) thickness and unassociated with left ventricular hypertrophy, was observed in 8 cases, all of which were cases of panlobular emphysema.

Lymphatic patency was maintained in three of the eight cases of PLE in which there was right ventricular hypertrophy. Among these was one with moderate CLE complicated by severe bronchopneumonia. The single instance of severe "pure" CLE in which suppurative pneumonia occurred despite patent lymphatics was one in which mitral stenosis was also present.

TABLE V

Lymphatics in Emphysema and Bronchitis (37 Cases)

Lymphatic patency	Comparison of centrilobular emphysema (CLE) and panlobular emphysema (PLE)						Bronchitis without CLE or significant PLE	
	Severe CLE		Moderate CLE		Mild or absent CLE			
	Open lymphatics	Closed lymphatics	Open lymphatics	Closed lymphatics	Open lymphatics	Closed lymphatics	Open lymphatics	Closed lymphatics
Severe PLE	—	2	—	—	3	2 + 2*	—	—
Moderate PLE	—	2	—	1	2	3	—	—
Mild or absent PLE	—	3	—	1	—	—	—	—
Without CLE or significant PLE	—	—	—	—	—	—	5†	11

* Destructive PLE with cyst formation.

† Complicated by anasarca + renal shutdown (2 cases), neurogenic pulmonary edema, subsiding pneumonia + arteriosclerotic heart disease, and lymphatic carcinomatosis.

FIG. 12. Centrilobular emphysema showing selective retention in emphysema saccules of India ink gelatin mixture dripped into bronchi before fixation (lung fixed in inflation).

In an experimental demonstration of impaired lymphatic drainage in centrilobular emphysema, a small quantity of warm 5 percent gelatin solution (about 200 cc) was introduced into the main bronchus of a freshly inflated, mildly emphysematous lung in the negative pressure chamber and followed at once by a drip of 2,000 cc of buffered 10 percent formalin to which 50 cc of India ink had been added. The lung was processed in routine manner, and slides were stained with hematoxylin and eosin. Little or no blackening was encountered in normal alveolar tissue, while a distinct, often thick, coating of black homogeneous material was found to line the saccules of centrilobular emphysema (Fig. 12). Deposits of anthracotic pigment were easily distinguished by their granular character.

Relation of Lymphatics to Bronchitis

The frequent association of chronic bronchitis with emphysema and the controversy over the nature of that association lend more than casual interest to comparisons of lymphatic patency in the two disorders. For this purpose, data on lymphatics were assembled in 16 cases of clinically severe chronic bronchitis unassociated with CLE or significant degrees of PLE.

The lymphatics were closed in 11 cases, a majority of which were associated also with the presence of asthmatoid wheezing. Lymphatics were patent in the remaining 5 cases, all of which were associated with conditions strongly favoring lymphatic widening such as anasarca and renal shutdown comlicating amyloid nephrosis or left-heart failure with renal infarcts, neurogenic pulmonary edema complicating intracranial hemorrhage, subsiding pneumonia in a case of left-heart failure, and lymphatic obstruction by metastatic carcinoma (Table V).

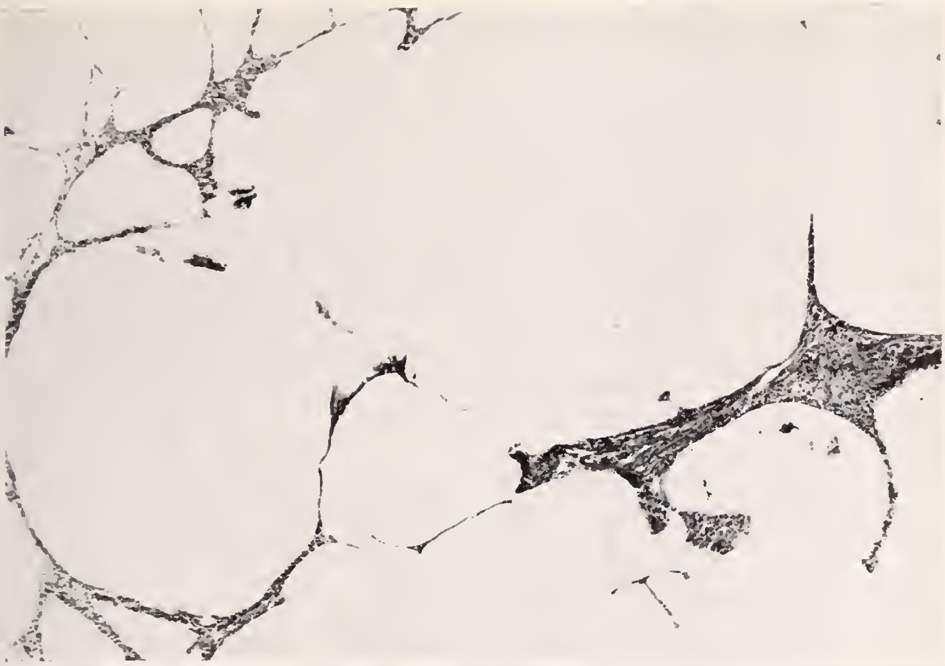

FIG. 13. Centrilobular emphysema showing abnormal distensibility of alveolar tissues on inflation in presence of sump disease.

Discussion

Far from being passive drainage canals, lymphatic vessels have been shown to act in close concert with the blood supply in maintaing a steady flow of protein-rich nutrient fluid about tissue cells (10–13). Many lines of evidence indicate that lymphatics function as "force pumps," possessing numerous "inlet valves" at intercellular junctions through which fluid is sucked from the interstitial spaces and propelled to larger collecting lymphatics, eventually to rejoin the circulating blood (14). The pumping mechanism is actuated by pressures conveyed to lymphatics from contracting muscles or, in the case of the lung, by rhythmic respiratory pressures, aided measurably by the pulsations of contiguous arteries (2, 10, 11, 15, 16).

There is reason to believe that the movements of fluid within the interstitial space are directed ordinarily, not by preformed open channels but by "capillarity," the fluid being conducted in a film around and between connective tissue filaments. By reason of their great number, these present a relatively enormous surface area (17).

The alveolar septum is ideally adapted for its function as a gas exchanger by the thinness of its surface epithelium and the closeness of the underlying capillaries. In many places, the interstitium between alveolar wall and capillary is undetectable ultramicroscopically, and alveolar and capillary basement membranes appear to fuse. Elsewhere they are separated by a narrow intermem-

TABLE VI

Lymphatics in Emphysema

Lymphatic patency	Comparison of lymphatic patency in CLE and PLE in relation to suppurative pneumonia and right ventricular hypertrophy*																	
	CLE (Severe)						CLE (Moderate)						CLE ±					
	SP		SP + RVH		RVH		SP		SP + RVH		RVH		SP		SP + RVH		RVH	
	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed	Open	Closed
PLE (Severe)	—	1	—	—	—	1	1†	—	—	—	1†	1	—	—	—	1‡	1	1 + 1‡
PLE (Moderate)	1§	1	—	—	—	—	—	—	—	—	—	—	—	1‖	—	—	1	—
PLE ±	1†	1	—	—	—	—	—	1‖	—	—	—	—	—	—	—	—	—	—

* Unassociated with left ventricular hypertrophy.

† With congested lung or nonsuppurative pneumonia.

‡ Destructive PLE with cyst formation.

§ Associated with chronic lymphedema.

‖ With endotoxin shock.

Symbols: SP (suppurative pneumonia); RVH (right ventricular hypertrophy) (6 to 8 millimeters), PLE (panlobular emphysema), CLE (centrilobular emphysema), and ± (mild or absent emphysema).

branous space (18–23) which, presumably, is filled largely with jelly-like matrix material. Fine connective tissue fibrils lie embedded in the homogeneous substance of the basement membranes or within the matrix material of the intermembranous space. The network of fibers, which extends about blood vessels and conducting airways, provides support for the lymphatic vessels and, theoretically, conduits for interstitial fluid.

The expanded interstitium contained within the fibrous covering sheath of the respiratory bronchiole and its artery, by reason of its location at the apex of a system of branching alveolar ducts and sacs, constitutes a physiological catch basin or sump toward which the numerous microcurrents of interstitial tissue fluid and lymph converge under the influence of a negative pressure gradient of considerable magnitude, which may be as much as 15 mm Hg subatmospheric (24). The onward flow of interstitial fluid depends on the adequacy of the siphoning force of the sump lymphatics. Because bronchiole, artery, and lymphatic vessels are held together in close contact by their common outer, confining sheath, the pulsation of the artery is effectively converted to the use of the lymphatic pump mechanism.

The regularity with which a fairly large lymphatic channel can be demonstrated in relation to respiratory bronchioles identifies the importance of these sites in relation to the dynamics of fluid transport within the lung, not only in connection with tissue fluids, upon which this report is chiefly centered, but with fluids absorbed from air passages as well. One does well to remember that Macklin (2), who fathered the sump concept, proposed renaming these bronchioles 'absorptive bronchioles' on the basis of his supposition that they were miniature lymphoepithelial organs, specifically developed to pump the watery part of redundant alveolar fluid into lymphatic vessels following completion of its surfactant role. In a larger perspective, the pulmonary sump may be regarded as essentially a conduction node capable of giving direction both to interstitial and alveolar fluid. In this light, the scope of its physiological importance far exceeds that contained in Macklin's original concept.

The efficacy of the sump depends not only on negative interstitial pressure generated by lymphatic siphonage but, perhaps, in greater measure, on the additional negative pressure imparted to the bronchiolo-arteriolo-lymphatic compartment by expanding alveoli (25).

The technique I employ in perfusing the inflated lung with fixing solution provides a working model of fluid transport from airways and interstitial tissues into the lymphatics. The degree of lymphatic patency demonstrated histologically mirrors to a large extent the operation of the lymphatic pumping mechanism in health and in disease.

The proportion of widely open sump lymphatics was found to be increased, for example, in inflammatory and congestive disorders, and they were widened in exceptional degree when these disorders existed in combination (see above). The predominance of closed lymphatics in the shock syndrome accounts, perhaps, for the predisposing effect of shock on the development of bacillary necrotizing pneumonia (26), an idea anticipated by Laurenzi's observation of the profound lowering of pulmonary resistance to airborne microorganisms in SPF mice that were harboring intestinal infection with endotoxin-producing bacteria (27).

The higher proportion of closed lymphatics in cases of chronic bronchitis, with or without centrilobular emphysema (Table V), is of interest in relation to the role of bronchitis in the pathogenesis of emphysema. The observations suggest that the underlying basis of atrophy and rupture of alveolar septa in CLE is *sump failure* engendered by a combination of factors associated with bronchitis.

Of these, one of the most important is chronic cough, especially in the presence of increased airway resistance. Transpulmonary pressures build up during successive coughs to a higher maximum in patients with bronchitis than in controls, reaching levels of 180 mm Hg (28). The high pleural and high alveolar pressure during cough tends not only to squeeze blood out of the pulmonary capillaries and to obstruct pulmonary bloodflow but must surely have a corresponding effect also on the transport of fluid in the interstitial tissue and lymphatic system of the entire lung.

A persuasive evidence of the importance of cough in the pathogenesis of centrilobular emphysema is the common observation that CLE is located predominantly in the upper parts of the lung and particularly in the apices. The same tendency exists in the evolution of cavities in tuberculosis, a condition also characterized by cough. The "lighting up" of normal apices on command to cough when a healthy chest is examined fluoroscopically is familiar to radiologists and is probably attributable to the greater muscular development of the lower thorax and abdominal wall, particularly in males. Accordingly, the forceful contraction of the thorax during vigorous cough (or when one tenses his muscles in the Valsalva maneuver or while lifting a heavy object) is exerted chiefly on the lower parts of the lungs, especially in the region of the diaphragm. The upper lobes, being less well defended by external musculature against high transpulmonary pressures, are momentarily hyperinflated during cough. In some instances, an actual bulge can be seen or felt in the supraclavicular fossae. The "lighting up" phenomenon can in fact be observed when the patient lies supine during fluoroscopy (personal correspondence, Maurice J. Small*), which speaks against the explanation given commonly that it results from the emptying influence of gravity on the apical blood vessels.

Cough-induced lymphatic blockage, sufficiently prolonged, must inevitably exact its toll. There are numerous clinical and experimental evidences that blocking the outflow of tissue fluid into lymphatic vents can seriously derange the internal milieu of tissues and lead to metabolic injury of cells. The harm of stagnant fluid lies in its capacity to weaken cell vitality, invite infection (10, 11, 29), and fill the interstitia with unwanted fibrous tissue (10, 11). The interstitial accumulation of collagen, which has been demonstrated ultramicroscopically in some alveolar septa of emphysematous lungs (30, 31), and the atrophy and the lack of significant fibrosis in others (32) undoubtedly result in much inhomogeneity of tensile strength. Such altered septa are probably especially vulnerable to inflationary stresses, particularly during repeated cough, and give rise to fenestrae (33), followed by their total disruption. The saccules and bullae that arise owe their spherical form to the resultant pressures within the expanding lung, which can be demonstrated readily if an isolated lung is subjected to inflation after irregular gross tissue defects have been created with a bronchoscopic rongeur introduced through the main bronchus (34).

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Without doubt many additional factors besides cough contribute to sump failure and the subsequent development of CLE in chronic bronchitis. Mucociliary insufficiency, perhaps, by slowing the migration of the moving film of alveolar surfactant fluid toward terminal bronchioles (35), permits its load of unwanted foreign particles to become sidetracked at sump sites and eventually to enter sump tissue along with alveolar fluid in process of absorption (2, 36–38).

Air pollutants concentrated many hundredfold by adsorption to certain types of particles (A. Goetz as cited by Hatch and Gross [36]) may work havoc with sump tissue, destroying the usefulness of its surface epithelium as a filter and introducing inflammation and fibrosis (Fig. 10). It is hardly cause for surprise that so much attention has been given to this aspect of alveolar clearance in the pathogenesis of CLE. What must be remembered is that the primary event in CLE is basically a process of attrition or atrophy (32, 33, 39), whether produced by halt of egress of tissue fluid from alveolar septa into sumps, as suggested by this study, or by failure of ingress because of reduced blood flow, as suggested by experimental studies of widespread pulmonary embolization (40), or by the common action of cough on both fluid portals.

Once initiated, emphysema will tend to deplete any residual sump activity to extremely low levels if not to the point of complete extinction. In this sense, emphysema, especially CLE, carries within itself the mechanism of its own propagation, ending only when the entire lobule has undergone cystic atrophy.

Another pathogenetic link between bronchitis and CLE may be the process of retrograde lymphatic spread of infection to sump areas during acute exacerbations of bronchitis, engendering, in turn, acute interstitial bronchitis and subsequent interstital fibrosis. It is not unlikely that recurrent lymphangitis of this sort progresses to chronic lymphedema manifested in the form of subpleural and paraseptal fibrocystic disease, i.e., "honeycomb lung" or large subpleural blebs and bullae.

Focal atelectasis secondary, perhaps, to mucus impaction in bronchi, a common complication of bronchitis, must not be overlooked as a factor predisposing the centrilobular region to overdistention. In atelectatic tissue, one often encounters widely distended bronchioles and alveolar ducts. The picture is not unlike that of the atelectatic lung of premature infants and, particularly near the pleural surface, can be attributed to absence of external support by an ambient cushion of uniformly inflated parenchyma.

A contributory factor may be the continued presence of alveolar surfactant in the aerated parts of alveolar ducts. The formidable imbalance of distending pressures during breathing and cough may stretch these dilatable parts to the point of rupture, especially where their supporting framework has undergone atrophy.

Sump failure, postulated as the initiating factor in CLE, can, with equal logic, be held responsible for progression of CLE to formation of large cysts and bullae. The process of septal attenuation, which, I believe, owes its origin principally to impaired circulation of interstitial nutrient fluid, spreads to more and more alveoli as lymph siphonage ebbs in the overinflated tissue. Whereas, previously, the sumpward movement of tissue fluid was halted or reversed for brief intervals whenever the patient coughed, it is now hindered constantly as the negative pressure gradient is abolished within the interstitia. Loss of gradient, which begins when alveoli have become permanently overstretched, is perpetuated by loss of radial traction of inflatable alveoli as their attachments are torn loose and indeed reversed altogether when uninterrupted high positive pressure develops in emphysema saccules as a result of air trapping. The altered pressure dynamics resulting from CLE interfere also in considerable measure with local blood flow and, therefore, with the flow of nutrients to the damaged tissue which might otherwise aid in repair.

As Fishman (41) has remarked, "Pathologic anatomy has allowed the clinician and the physiologist to conceal, under the cloak of 'emphysema,' a variety of disease processes." The observations reported here are hopefully a promise of what future studies of pathogenesis may bring to light in establishing "clear-cut definitions and classification as a basis for communication." The need for careful distinction between CLE and PLE is still far from universally recognized. That they are basically separate diseases has nonetheless been convincingly set forth by a number of investigators (42–44).* In addition to different rates of lymphatic patency, patients with PLE in this series were notably free of the tendency to suppurative pneumonia (in the absence of cystic degeneration) but prone to right ventricular hypertrophy (Table VI). One might theorize that generalized though mild reduction in radial traction, while sufficient to raise pulmonary vascular resistance of an entire lung, produces comparatively little loss of negative pressure gradients between alveolar septa and sumps and between sumps and lymphatic run-off channels. On this basis, the frequent combination of PLE and CLE and the progressive vanishing of tissue in cases of PLE uncomplicated by CLE can be attributed to multifocal or generalized severe impairment of the sump mechanism, induced either by coincidental chronic bronchitis, by continuing dissolution of radial traction, or by their combination.

A simple, yet striking demonstration of the degree of sump impairment which exists in CLE is provided by the experiment in which an India ink gelatin mixture run into the bronchus of the inflated lung was trapped selectively in emphysema saccules despite vigorous perfusion with the usual large volume of fixing solution dripped through the same bronchus (Fig. 12). One might therefore infer that the deposits of anthracotic pigment which are so common within the saccules of CLE reflect the same kind of entrapment in the living patient, hence represent the *result* of CLE rather than one of its causative factors (46).

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* The role, if any, of alpha$_1$-antitrypsin deficiency in the pathogenesis either of CLE or PLE is controversial. The destructive lesions in antitrypsin deficiency are generally diffuse and basilar. Injury of the alveolar capillaries has been suggested as the primary lesion, induced by the action of proteolytic enzymes released from leukocytes sequestered in the lungs in inflammatory foci (45).

The cardinal importance of lymphatic siphonage in determining the outcome of pulmonary infection is reflected in comparing the data on non-suppurative and suppurative pneumonia in the present series. In the former (Table III), the lymphatics were patent in 23 of the 31 cases. In cases of suppurative pneumonia, the situation was reversed, all but five of the 18 cases manifesting evidences of inadequate lymphatic transport. In nine cases, the sump lymphatics were closed; in three others with open lymphatics there was reason to suspect blockage resulting in chronic lymphedema (bullous emphysema, honeycomb lung), and in a fourth there was mechanical blockage by metastatic cancer (Table IV). Taken solely from the standpoint of emphysema, suppurative pneumonia existed in seven out of 25 cases of severe or moderate CLE with or without PLE and in only one of 18 cases of severe or moderate PLE unassociated with CLE or cystic degeneration. The patient with CLE is beset, therefore, with the constant problem of restricted lymph flow in the lung, a factor of importance in relation to the tendency of CLE to progress in severity and to predispose to secondary infection of highly lethal character.

Summary

Efforts to define the role of pulmonary lymphatics in the evolution of lung disease have been largely thwarted because of the inadequacies of available technical methods. In this report a method is described by which the pulmonary lymphatics are made visible under the microscope in a form closely approximating their condition in the living patient. The principle of the method, which is well suited to routine application in the autopsy room, consists in perfusing the lung with slow intrabronchial drip of formalin solution in order to harden the lung while maintaining its inflated state under gentle negative pressure. Observations thus obtained in a series of 166 autopsy cases provided a number of interesting insights into the pathogenesis of several types of lung disease including emphysema.

One of the results of this study was the demonstration that the fixing solution passes rapidly from alveoli into lymphatics when the negative pressure is well within the physiological range. This finding suggests that a physiological pumping mechanism depending largely on negative pressure is responsible for the normal transport of tissue fluid from the alveolar interstitium into the lymphatic siphonage apparatus. The anatomical representation of the pumping mechanism, as suggested by these studies, is the interstitial space at the apex of pulmonary lobules, where bifurcating respiratory bronchioles, small arteries, and lymphatic channels are held closely together within a common supporting sheath of fibroelastic tissue. This structural unit, in which a node of relatively high negative pressure is postulated to exist in life, may be designated as the pulmonary 'sump.'

The efficiency of the pumping apparatus of the sump may be the crucial factor in maintaining the homeostasis of the interstitial environment of the alveolar septa. Impairment results when the sump tissue becomes thickened by inflammation and fibrosis induced by chronic bronchial infection and continued inhalation of irritant dusts and fumes. The hindrance to movements of tissue fluid in such cases can be assumed to cause stagnation of metabolic waste products and other harmful substances in alveolar interstitial tissue, followed by progressive atrophy and eventual dissolution of the alveolar framework, especially nearest the apex of the lobule. Then, as a consequence, the stress of oft-repeated cough can be sufficient to rupture the walls of weakened alveoli to preduce centrilobular emphysema.

The importance of lymphatic run-off in clearance of pulmonary transudates and exudates, well established by numerous physiological studies, was reaffirmed anatomically in the form of unusually wide lymphatics in the lungs of patients dying of cardiac failure or nonsuppurative pneumonia. Conversely, failure of the lymphatic clearance mechanism seems to underlie the development of suppurative pneumonia complicating shock states or centrilobular emphysema, in which the lymphatics generally appeared narrowed or obliterated.

Chronic lymphatic blockage in the form of chronic lymphedema of a type observed in other tissues, yet ordinarily unrecognized in lungs examined by conventional methods, was brought to light clearly in several cases of this series.

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Papilledema with Acute Viral Infections of the Brain*

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Abstract

Six patients with papilledema in probable viral meningo-encephalitis, the etiology of which was proven in three, are presented. Four of these were obtained from a review of 100 charts of patients with probable viral disease of the brain—an incidence of four percent. The available English language literature indicates that papilledema can occur with most viral meningo-encephalitides, but most often when Herpes simplex is the responsible virus. The differential diagnosis of papilledema in virus infections includes optic neuritis and retinal vein thrombosis, and the treatment of increased intracranial pressure in some viral illnesses with steroids, glycerin, urea, or mannitol is mentioned. Lumbar puncture in patients with papilledema due to virus invasion of the brain is thought to be an indicated procedure.

The signs and symptoms produced by acute viral infections of the nervous system are usually caused by: 1) direct viral invasion of the brain, spinal cord, nerve roots, peripheral nerves and their coverings; or 2) activation of latent viruses which do this; or 3) various delayed antigen-antibody immune reactions; or 4) increased intracranial pressure. With the exception of the recent, now rather extensive, literature concerning neuroinfection with Herpes simplex virus, this fourth mechanism, has been generally ignored or minimized in most previous reports and discussions of viral infections of the brain.

The author recently saw a patient with papilledema whose only significant laboratory abnormality was a spinal fluid which contained 250 white blood cells, nearly all lymphocytes. All attempts to find a definite etiology for this were unsatisfactory, and the follow up course of this patient indicates that a viral infection was probably responsible. If lumbar puncture had not been performed in this patient with papilledema, the probable viral etiology of her condition would perhaps have never been recognized.

To determine how often papilledema occurs in viral meningo-encephalitis, 100 charts of patients hospitalized at The Mount Sinai Hospital (New York) with a definite or probable diagnosis of viral meningitis, encephalitis, or meningo-encephalitis were reviewed. Four of these patients had papilledema some time in their hospital course. An additional patient, also hospitalized at Mount Sinai, with papilledema and infectious mononucleosis, has been previously reported (1). The reports of these six patients, five from Mount Sinai, and one personally observed by the author elsewhere, form the substance of this communication. The pertinent literature will also be reviewed.

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Materials and Methods

The 100 charts which satisfied certain criteria for the diagnosis of viral meningo-encephalitis were obtained from a review of about 400 charts bearing this diagnosis. These charts go back over 20 years. The criteria for acceptance of the diagnosis as correct for this study consisted essentially of cerebrospinal fluid lymphocytosis with normal sugar content, in the absence of any condition, other than a viral infection, which could produce this. Unless a viral etiology was definitely established, patients who received prior antibiotic therapy, which may have resulted in a partially treated bacterial meningitis or patients in whom subsequent studies or courses or admissions indicated another cause for their spinal fluid lymphocytosis (such as demyelinating disease, tuberculosis, or sarcoidosis), have been excluded from this study. Two children with initial spinal fluid predominance of polymorphonuclear leukocytes shortly after the onset of acute symptoms, but with subsequent detection of a viral etiology and spinal fluid lymphocytosis, have been included. It is acknowledged that many other patients with initial spinal fluid pleocytosis with polymorphonuclear cells or with lymphocytosis on antibiotic therapy actually may have had virus infections, and they have been excluded from this study.

A definite viral etiology was indicated in the charts of only 16 patients. This very low figure is probably due to the lack of inclusion in the charts of subsequent serological study of convalescent phase serum, which was often obtained after discharge, and to the fact that the study includes some patients hospitalized as far back as 1950.

Case Reports

Case No. 1: A 39-year-old woman was hospitalized when her physician noted bilateral papilledema. Several weeks earlier she had a febrile illness, the symptoms of which included malaise, generalized weakness, and pains in her back and legs. After one week she began to develop severe headaches, nausea, and vomiting. There were no other neurologic symptoms. There had been no exposure to known contagion.

Her physician saw her several times during the early phases of this illness. No abnormalities were noted during these physical examinations, except low grade fever. Routine laboratory outpatient studies were normal.

After two to three weeks the fever subsided, and the headaches improved. For the first time, however, papilledema was noted on examination and hospitalization advised.

The detailed general and neurological examinations were completely normal except the fundi. There were bilateral blurring of the disc margins, elevated discs, significant venous distention and several petechial and splinter hemorrhages in both eyes, right more than left.

Routine laboratory studies were again normal. The electroencephalogram, skull roentgenograms and brain scan were all unremarkable. Careful lumbar puncture obtained crystal clear spinal fluid, with an opening pressure of 190 mm water and a closing pressure of 170 mm water. This fluid contained a protein content of 40 mg percent and a sugar of 50 mg percent, but had 250 white blood cells, 90 percent of which were lymphocytes. Torula preparations, routine bacterial, tuberculous, fungal and viral cultures were subsequently reported negative, as was a V.D.R.L. Several heterophile titers, P.P.D. and fungal skin tests, electrocardiogram, serum amylase, pharyngeal washings and stool cultures for virus, and convalescent serum titers for neurotrophic viruses were either normal or negative.

The patient's course was characterized by continued improvement. She became asymptomatic, and the examination remained normal, except for the fundi, which showed improvement. Because she was improving, contrast neuroradiologic study was not performed. A second lumbar puncture, performed 10 days after the first, was normal, except for 41 white blood cells, 84 percent of which were lymphocytes.

Her fundi were normal in a follow-up neurologic examination two months later, and she has remained well in the 2 years since papilledema was first noted. (This patient was not hospitalized at Mount Sinai.)

Case No. 2: A seven-year-old boy was hospitalized in 1970. Twelve days earlier he began to complain of generalized headache, which progressed in severity. Ten days before admission his right eye was noted to turn in, and a head tilt, with the occiput towards the left shoulder, was noted. An ophthalmologist noted bilateral papilledema and he was referred to a neurologist and hospitalized. There had been no fever or rash during this acute illness.

The general physical examination was unremarkable. On neurologic examination, the patient complained of diplopia on direct forward gaze. There was a tendency for the right eye to deviate in, but no definite extraocular palsy was noted. There was a head tilt to the left. Frank papilledema was present in both eyes.

Routine laboratory studies were normal. Skull roentgenograms revealed the sutures to be slightly prominent, but were not definitely abnormal. An electroencephalogram was diffusely abnormal. The cerebrospinal fluid was crystal clear, but had an opening pressure of 340 mm water and contained 125 white blood cells, nearly all lymphocytes. Sugar, protein, and routine cultures were normal. Bilateral brachial and a carotid arteriogram were normal. Serum Vitamin A and carotene levels were normal, as were many other studies.

An echovirus, Type 3, was isolated from spinal fluid.

The patient's hospital course was afebrile throughout. The fundi slowly improved, and all symptoms subsided. Repeat spinal fluid examinations and a follow-up electroencephalogram also improved. Neurologic examinations after discharge were normal.

Case No. 3: A five-year-old girl was admitted to the Neurology Service after a grand mal siezure. She had previously been well and had no other neurologic symptoms. There was no fever at any time.

Her initial general physical and neurologic examinations were normal. An electroencephalogram had bilateral slow waves, and an initial lumbar puncture contained 18 lymphocytes, but was otherwise normal. Other laboratory study was normal.

There were no further seizures, but she was noted to be drowsy (not felt to be related to anticonvulsants) and developed a significant bradycardia. The pediatric neurologist, who examined her shortly after admission, noted a change in her fundi, in that the disc margins were becoming blurred. A repeat lumbar puncture resulted in fluid still under normal pressure, but now containing 36 lymphocytes. Intravenous mannitol therapy resulted in prompt improvement, which was maintained.

Attempts to determine the cause of her spinal fluid lymphocytosis or transient (probable) increased intracranial pressure were unsuccessful. She was neurologically normal and had a normal electroencephalogram in the Neurology Clinic two months later.

Case No. 4: A 52-year-old woman was transferred from another hospital for further evaluation of fever of three weeks duration. She had also had frontal headaches and initially mild upper respiratory symptoms. She was transferred when spinal fluid analysis revealed 102 lymphocytes and a protein of 110 mg percent. Spinal fluid sugar, pressure, and other laboratory studies had been normal.

On admission her temperature was 100° F, and she was lethargic. The optic disc margins were blurred. There were no other significant abnormalities on general physical or neurologic examinations.

Her course in the hospital was characterized by initial progression. Her temperature rose to 103° F, and she developed increased blurring of the disc margins, venous engorgement, downward nystagmus, and meningeal signs. Electroencephalograms revealed bilateral abnormalities. Four further lumbar punctures resulted in clear fluid, always under normal pressure, with cell counts falling from 91 white blood cells to 28, mostly lymphocytes. Spinal fluid protein fell from 90 to 64 mg percent. Sugars, all cultures, skin tests, Torula preparations, serologic tests of spinal fluid and blood for viruses, syphilis, and all other laboratory studies were normal or negative.

After three weeks of hospitalization she began to improve and her fundi reverted to normal. She did develop some Parkinsonian features, which persisted after discharge.

Case No. 5: A 14-year-old girl had lethargy, nausea and vomiting, headaches, and generalized malaise for two weeks. These symptoms fluctuated in intensity initially but all became quite severe two days before admission, at which time she also complained of photophobia and eyeball tenderness. There was no fever, rash, or exposure to known contagion.

On physical examination she was lethargic and found to have bilateral papilledema with some meningeal signs.

Spinal fluid was somewhat opalescent, but with a normal pressure (180 mm of water). The fluid contained 373 white blood cells, 80 per cent of which were lymphocytes, but a normal protein and sugar content. Three follow-up lumbar punctures revealed clear fluid with the cell count falling to 73 lymphocytes. Routine, fungal and tubercular cultures were all sterile. Virus isolation techniques of the spinal fluid were not satisfactory, but serologic study did reveal a significant rise in neutralizing antibody titer to Coxsackie B, Type 3.

An initial electroencephalogram had bilateral slowing; a repeat was normal. Skull roentgenograms, brain scan, routine blood counts, sedimentation rates, and a variety of other laboratory studies were normal.

She remained afebrile throughout her 18 days of hospitalization. Her course was characterized by continued improvement, and she soon became asymptomatic. The fundi started to improve by the fourth hospital day, and she was discharged with only minimal blurring of the optic discs.

Case No. 6: This case has been reported previously (1). An 18-year-old boy had severe headaches for two weeks and horizontal diplopia for ten days. He was hospitalized when bilateral papilledema was found on examination. There were no systemic symptoms, and the results of general physical examination were normal. In addition to the papilledema, which included some hemorrhages, the neurologic examination also suggested some paresis of abduction of the right eye, but was otherwise intact. Two spinal fluid examinations were normal, except for an elevated pressure in the first lumbar puncture. Skull roentgenograms, brain scan, electroencephalogram, retrograde brachial arteriogram, and a pneumoencephalogram were all normal. This patient had taken some Vitamin A capsules for acne, but a serum Vitamin A level was normal.

There were atypical lymphocytes in peripheral blood smears, and a heterophile titer of 1:112 was obtained. Improvement occurred rapidly.

Discussion

Four percent of a selected group of 100 patients with viral meningo-encephalitis were noted to have papilledema at some time during their hospitalizations. One meningo-encephalitis was related to Coxsackie and another to echovirus. In two other patients, the etiologic virus could not be identified. In addition to these four patients, two other patients with papilledema and viral infection of the brain have been presented. Infectious mononucleosis was involved in one of these latter patients.

An infectious process might not have been considered in these six patients, since only two exhibited meningeal signs and only one had fever during hospitalization. One other had been febrile before hospitalization. It was only lumbar puncture, performed despite the presence of papilledema, that suggested an infectious etiology for five of these patients.

Much of the current literature concerning increased intracranial pressure in viral diseases of the brain relates to Herpes simplex encephalitis (2–5), which is said to cause papilledema in ten percent of cases (6). The pathological changes due to increased intracranial pressure in this disease have been well described (7), and the attempts of therapy by means of reduction of intracranial pressure emphasized (4). The one patient with Herpes simplex encephalitis in the current series was an infant who died with a fulminating illness, but with normal fundi during life.

Increased intracranial pressure and papilledema occur much less often in other viral meningo-encephalitides. Two Scandinavian monographs of 102 (8) and 238 (9) patients with a variety of probable viral meningo-encephalitis do not even mention papilledema as occurring in any of these 340 patients. Another study (10) of 68 patients with viral encephalitis, many with Herpes simplex, reveal that 19 or 28 percent had papilledema. Some references to the observation of papilledema in other specific viral infections of the brain have appeared. These, however, have not been emphasized before.

Measles: Much has been written about the encephalitis of measles, at least in previous years. Ford (11) in 1928 collected 113 cases of neurologic complications of measles in the literature and added 12 patients of his own. One patient in the literature had "chocked discs" and one of his patients had "blurred optic discs and dilated veins". There were also two patients with "optic neuritis" and one case of "papillitis" in the literature. In addition to optic neuritis, Ford also described retinal vein thrombosis in measles. Both conditions can cause confusion and may be mistaken for papilledema. This would apply to the evaluation of fundiscopic changes in other viral diseases of the brain.

Appelbaum et al (12), reported "congestion or blurring of the optic discs" in two of 74 patients with measles encephalomyelitis, and felt that the cerebrospinal fluid was frequently under increased pressure. Ford (11) reported an elevated spinal fluid pressure in 12 of the 18 patients in whom this pressure was measured.

In an exhaustive review of "parainfectious encephalomyelitis" published in 1956, Miller et al (13) collected 630 cases of measles encephalitis in the world literature. Eleven of these patients had papilledema. Three of the 85 patients with neurologic complications of measles reported by Tyler (14) had "blurring of the optic discs" and "hyperemia" of the fundi. Five other patients also had a retrobulbar neuritis. Tyler (14) also described a "toxic encephalopathy," a late complication of measles, which produced papilledema in three patients with no changes in their spinal fluid protein or cell contents. Eyeground changes were not mentioned in any of the 170 patients with measles meningo-encephalitis presented by Boughton (15), although he felt that cerebrospinal fluid pressure was usually elevated, or in any of the 61 patients with measles encephalitis reported by LaBoccetta and Torway (15), or in the 50 cases described by Rozycki (17).

Rubella: The neuropathology of German measles encephalitis may differ from that of other viral encephalitides (13). Of 74 cases of rubella encephalitis collected from the literature by Miller et al (13), four were said to show papilledema. This was due to optic neuritis in two. Cerebrospinal fluid pressure was thought to be elevated in half of the patients with rubella encephalitis. Encephalitis is a relatively rare complication of rubella, and no large series of cases has been found in the literature. One fatal case out of a group of five patients with rubella encephalitis did have blurred discs and an elevated spinal fluid pressure (18).

Varicella: The encephalitis of varicella is somewhat more frequent than that of rubella. Cerebellar involvement is particularly common. Of 121 cases of chicken pox encephalitis collected from the literature by Miller et al (13), only two had papilledema. As best as can be determined, the eyegrounds were normal in the 59 patients with varicella encephalitis reported by Appelbaum et al (19), and only in one patient of the 38 described by Boughton (20) were the "retinal veins rather full." Of 57 cases of Johnson and Milbourn (21), two were said to show blurred discs. These authors felt that most fatal cases of varicella encephalitis would show cerebral edema at autopsy, and used corticosteroids and mannitol to combat this. Steroids and urea were also employed by Jenkins (22) in an attempt to treat the cerebral edema of varicella encephalitis. The hemorrhagic encephalopathy which occasionally complicates chicken pox may result in retinal hemorrhages producing difficulties in the evaluation of papilledema (23).

Mumps: Mumps virus usually produces an aseptic meningitis rather than an encephalitis, and the former rarely causes papilledema. Two of the 27 cases of mumps encephalitis collected from the literature by Miller et al (13) showed papilledema. Fundiscopic changes, even minimal ones, were not mentioned in the reports of 50 patients (24), 45 patients (25), 39 patients (26), 51 patients (27), and 64 patients (28), all with mumps meningo-encephalitis. Although coma and seizures have been described with mumps virus invasion of the nervous system, its usual neurologic manifestations would seem to resemble those of the enteroviruses and papilledema is quite rare.

Enteroviruses: Although two patients in the present series (Cases 2 and 5) did have papilledema related to enterovirus infection, the occurrence of fundiscopic changes is quite rare in the literature of these diseases. Most of the currently seen enteroviruses (polio is excluded from this discussion) usually do not produce neurological signs, other than those of meningeal irritation. They usually produce an aseptic meningitis, frequently in epidemic fashion.

One patient among the 64 with Echovirus Type 30 meningitis (29) showed "transient papilledema," as did one of the 196 children with Echovirus 9 meningo-encephalitis (30), and two of the 32 patients with Coxsackie B5 infections of their nervous systems (31). The author could not find any mention of any type of fundiscopic abnormality in any of the 1,556 patients with various Coxsackie or echovirus meningo-encephalitis collected from 14 published epidemiological reports of these conditions, in which the clinical manifestations were described in some detail.

There is a recent report of the use of glycerin to control the increased intracranial pressure associated with Echovirus 3 infection (32).

Miscellaneous viruses: Papilledema has been reported in patients with Herpes zoster encephalitis (33), California encephalitis virus infection (34), lymphocytic choriomeningitis (35), and other patients with encephalitis due to infectious mononucleosis (36, 37), other than Case 6 reported here and elsewhere (1). It is probable that still other viruses affecting the brain and its coverings have produced papilledema; but that reports of these have escaped the author's attention. The experimental production of aqueductal stenosis and other obstructions in the spinal fluid pathways and hydrocephalus in laboratory animals by intracerebral inoculation of various viruses (38, 39) offers one possible mechanism for papilledema induction by viruses (other than generalized increased intracranial pressure).

While infections of the nervous system can usually be suggested by fever, meningeal signs and other clinical features, this is not always the rule. Certainly, the diagnosis of virus infection of the brain would not have been made in Cases 1 through 5 in the present series without lumbar puncture. Only two of these six patients reported here had meningeal signs, which were mild, and only two had a history of temperature elevation.

It is the author's considered opinion that lumbar puncture may remain the only means of establishing or recognizing a viral invasion of the brain, even though this may occur in patients with papilledema.

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Epidemiologic Reconnaissance of Hypertension in the Bahamas:

With Special Reference to Depot Fat Composition and Its Implications in Relation to Cardiovascular Disease Prevalence

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Epidemiologic studies conducted in the past two decades have established associations between saturated fat intake and coronary heart disease, and the increased susceptibility to coronary heart disease in persons with elevated serum cholesterol, obesity, hypertension, and impaired carbohydrate tolerance (1). However, some populations have also been found which apparently demonstrate a disassociation of these risk factors and the occurrence of coronary heart disease (2). In several nonwhite populations in developing countries, an increased occurrence of hypertension is not associated with a high mortality rate from coronary heart disease (3). In spite of a diet high in animal fats, the Masai tribesmen rarely develop hypercholesterolemia or coronary heart disease (4). Diabetic East Africans rarely develop coronary heart disease (5).

Still another example of a disassociation between the presence of a risk factor (hypertension) and the occurrence of coronary heart disease appears to exist in the Bahama Islands, whose inhabitants share a West African-Anglo Saxon heritage with the people of other former English possessions in the Caribbean. Interest in the Bahamas was stimulated by the observations of local physicians that myocardial infarction rarely occurs in their Bahamian patients. This is a striking finding since hypertension and its sequelae are believed to be the major public health problems in the Bahamas.

The objectives of this preliminary study encompassed three specific areas of interest:

1. To obtain data on the prevalence of hypertension in a group of free-living Bahamian subjects.
2. To review available autopsy evidence of the degree of coronary atherosclerosis in hypertensive Bahamians dying at Princess Margaret Hospital in Nassau.
3. To determine whether fatty acid analysis of adipose tissue aspirates could yield clues as to whether dietary factors may reverse the effect of hypertension as a risk factor in coronary heart disease induction.

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Study Groups and Methodologies

Blood Pressure Survey: A group of 1488 free-living, Bahamian-born residents of the island of New Providence, 10–69 years of age, were enlisted into the study group. Subjects included children attending Government and private schools, parents of these children, hotel workers, policemen, and staff members of Princess Margaret Hospital in Nassau.

Blood pressures were taken from the right arm, in the standing position, after a 5-minute rest period. A Tycos aneroid manometer, standardized against a mercury manometer with a standard-size adult cuff was used. Readings were determined by the auscultatory method: systolic pressure was the first sound after obliteration of the brachial pulse; diastolic blood pressure was taken as the first muffled sound.

Autopsy Survey: The records of 1450 consecutive autopsies performed at Princess Margaret Hospital in Nassau between June 11, 1964 and December 6, 1967 were examined. Degree of atherosclerosis in the coronary arteries was graded as follows:

Minimal: plaques of atheroma not causing significant occlusion, i.e., less than 50% occlusion of the coronary vessels.

Moderate: plaques causing more than 50% occlusion.

Among 1450 persons autopsied, 126 were Bahamians with a clinical record of documeted hypertension who were selected for inclusion in this study.

Adipose Tissue Aspiration: Linoleic acid accumulates in adipose tissue only from dietary sources since according to present knowledge, it is not synthesized in vivo (6). Fatty acid composition of adipose tissue can accordingly serve as an index of dietary linoleate intake. Between December 1967 and February 1969, samples of adipose tissue were obtained from 85 Bahamian subjects, 11–59 years of age, utilizing an aspirating syringe technique (7). Sixty-eight subjects, 11–19 years of age, included Nassau school students and student nurse candidates. Subjects 20 years old and over were employees of the Princess Margaret Hospital Hypertension Clinic.

Immediately after adipose tissue aspiration, propyl gallate was added to the samples as an anti-oxidant. On arrival at the laboratory, samples were placed under nitrogen and stored at −20 degrees centigrade. They were submitted to gas chromatographic analysis using a column of diatomaceous earth with a diethylene glycol succinate liquid phase following formation of fatty acid methyl esters. Percentage composition of fatty acids was determined in duplicate samples by triangulation.

Results

In all age groups studied, the prevalence of hypertension in Bahamians was three to four times that found in Americans of corresponding ages (Table I). Twenty-six percent of Bahamians below 30 years of age had a diastolic blood pressure of 95 mm Hg or higher, compared to about 4% of Americans in similar age groups (8). In comparison to the inhabitants of St. Kitts, another Caribbean island, the prevalence of hypertension in Bahamians is four times greater in the

TABLE I

Comparative Prevalence of Hypertension in Bahamian, St. Kitts and United States of America Subjects

Percent of Subjects with Diastolic Blood Pressure of 95 mm Hg or Above

Age (Yrs.)	St. Kitts (9)	New Providence, Bahamas	U.S.A. (8)	Age
10-19	*	6%		
			1.4%	18/24
20-29	6.3%	26%		
			3.8%	25/34
30-39	17.6%	48%		
			10.1%	35/44
40-49	38.7%	65%		
			14.6%	45/54
50-49	*	62%		
			16.0%	55/64
60-69	*	73%		
			16.7%	65/74

* No data.

TABLE II

Autopsy Evidence of Coronary Atheroma in Nassau and the United States

Number	Subjects	Mean age
126	Hypertensive Bahamians 36%, Moderate or severe 64%, None or minimal	56 years
300	American Korean War Deaths (11) 77.3%	24 years
691	Deaths, Minnesota, 1947–1952 (10); 74%	

age group 20–29 years, about three times greater at ages 30–39 and two and one-half times higher at ages 40–49 (9).

The limited autopsy data related to hypertensive Bahamians obtained from Princess Margaret Hospital in Nassau is compared to two American studies in Table II. Standardized autopsy techniques were not utilized in these three sources of data, making comparisons subject to misinterpretation. However, 64% of the hypertensive Bahamians had no or very minimal evidence of coronary heart disease at postmortem (performed at a mean age of 56 years). By contrast, 74% of 691 autopsied individuals of an American community dying in middle age, had coronary atheroma (10), while 77.3% of young American soldiers killed in Korea (mean age 24 years) had "some gross evidence" of coronary atheroma (11). Although it must again be emphasized that the populations and techniques used in the three studies are not comparable by strict criteria, these results suggest that the prevalence of coronary artery disease atherosclerosis differs markedly in the United States and the Bahama Islands.

TABLE III

Adipose Tissue Fatty Acid Composition of Study Subjects by Age, Compared to Other Populations

Population Studied	14:0	14:1	16:0	16:1	18:0	18:1	18:2
Nassau, 68 subjects, 11-19 yrs.	2.65 ±1.73	0.68 ±1.39	21.65 ±4.67	4.36 ±2.51	10.69 ±6.69	46.39 ±11.7	12.81 ±5.2
Nassau, 17 subjects, 20-70 yrs.	0.95 ±0.52	—	19.85 ±3.5	5.18 ±1.62	5.30 ±2.09	54.53 ±7.45	13.28 ±3.60
New York (12), 19 men, 50-59 yrs.	2.0 ±0.63	—	23.7 ±0.68	5.8 ±2.11	3.8 ±1.3	53.2 ±4.56	9.7 ±2.45
New York (12), Anti-Coronary Club, 23 men, 50-59 yrs.	1.5 ±0.86	—	20.2 ±3.95	5.7 ±2.3	3.7 ± .47	46.4 ±5.1	18.9 ±4.54
New York (22), 22 subjects, 10-13 yrs.	1.8 ±0.2	0.4 ±0.1	19.4 ±0.6	7.0 ±0.3	3.4 ±0.4	54.1 ±0.9	12.8 ±0.6
Iowa (15), 6 subjects, 2½-17 yrs.	3.2	—	23.2	4.7	6.1	51.6	9.3

The adipose tissue composition of two groups of Bahamian subjects is outlined in Table III and compared to several groups of Americans reported in the literature. Bahamian adults have more oleic acid (C18:1) in their adipose tissue than Bahamian children (54.5% vs. 46.4%, $p < .01$). Bahamian children have more myristic acid (C14:0) in their adipose tissue than Bahamian adults (2.65% vs. 0.95%). Stearate levels did not alter significantly in the two Bahamian groups.

When compared to Americans, Bahamian adults have higher linoleic acid (C18:2) levels in their adipose tissue than has been reported in white middle-aged men in New York (13.28% vs. 9.7%, $p < .01$) (12), U.S. soldiers in Korea (9%) (13), and American Negroes 8.9% vs. 11.1) (14). Bahamian linoleic acid levels are midway between those of men on a diet high in polyunsaturated fat for 3 or more years and 'normal' American men, Table III (12). American white and Negro subjects have higher palmitic acid (C16:0) levels (22.6% and 23.7% respectively) than adult Bahamians (19.85%).

Limited data on adipose tissue composition of children exists with which to compare the younger Bahamian subjects. The largest series reported consists of Caucasian and Negro subjects in New York City (20), Iowa and Philadelphia (15, 16). The linoleate (C18:2) content of adipose tissue in Bahamian children is similar to the New York City youngsters, but higher than the Philadelphia or Iowa children (Table III). The stearate (C18:0) level of Bahamian youngsters is higher than the New York City and other groups ($p < .01$). Bahamian children also exhibited a higher level of palmitic acid (C16:0) than New York Caucasian children ($p < .01$), but did not differ significantly from Negro children living in the same area of New York. Both the Negro and Caucasian children in New York had higher levels of palmitoleic acid (C16:1) than Bahamian children ($p < .01$).

Discussion

The importance of hypertension as a risk factor in coronary heart disease in the USA is strongly supported by data from the Framingham study. Additional though less direct support comes from comparative mortality rates from coronary heart disease and hypertension in several countries compiled by the World Health Organization (Table IV) (17). Numbers in parentheses indicate the order of each country in mortality rate. The American nonwhite male and female lead in mortality rates from hypertensive disease. American nonwhite males are second only to American white males in mortality from ischemic heart disease. American nonwhite females have the highest mortality rate from ischemic heart disease among females of all other countries listed.

In nonwhite Americans, these mortality statistics lend support to the association of hypertension and ischemic heart disease. Furthermore, the male: female ratio for ischemic heart disease mortality in American Negroes is low (1.5:1) compared to that in white Americans (5:1) (18), suggesting that hypertension may be an especially important risk factor increasing coronary heart disease risk in Negro males and females.

Males of Japan, Taiwan, and Portugal have the second to fourth highest hypertensive disease mortality rates. However, they rank 21st to 23rd in male mortality rates from ischemic heart disease. Scotland and Finland, like the United States, have rankings in similar ranges in the mortality rates from both ischemic heart disease and hypertensive disease. The reasons for the disassociation in mortality rates for hypertension and coronary heart disease in the first three countries (Japan, Taiwan, Portugal) is not presently known. Although accurate mortality statistics are not available, cerebrovascular disease and congestive heart failure are said to be the principal causes of morbidity and

TABLE IV

Mortality Rates (Deaths/1000 Population) from Arteriosclerotic Heart Disease and Hypertensive Disease—By Age and Sex in Selected Countries—1958 (24)

Country	ASHD		HTD	
	(50–54 yrs.)			
	Males	Females	Males	Females
USA (white)	467 (1)	96 (4)	90 (10)	71 (15)
USA (nonwhite)	441 (2)	275 (1)	400 (1)	456 (1)
Japan	70 (22)	47 (15)	263 (2)	138 (2)
Taiwan	13 (23)	8 (23)	231 (3)	170 (3)
Portugal	80 (21)	37 (19)	116 (4)	86 (9)
Scotland	343 (7)	99 (3)	105 (5)	135 (4)
Finland	405 (3)	67 (10)	104 (6)	119 (5)

(Figures in parentheses indicate rank in mortality rate—No. 1 is the highest mortality rate.)

TABLE V

Percentage Composition of Adipose Tissue Fatty Acids in Several Racial Groups

	Population					
	14:0	16:0	16:1	18:0	18:1	18:2
Bahamians	0.95 ± 0.5	19.9 ± 3.5	5.18 ± 1.6	5.3 ± 2.1	54.5 ± 7.5	13.3 ± 3.6
Jamaicans (19)	8.4 ± 0.6	26.6 ± 0.8	10.1 ± 0.7	5.8 ± 0.5	37.7 ± 0.9	5.8 ± 0.6
Nigerians (19)	2.5 ± 0.18	28.7 ± 0.7	5.4 ± 0.6	8.0 ± 0.5	46.1 ± 0.7	8.0 ± 0.6
Bantu (21)	2.6 ± 0.4	22.3 ± 2.9	7.5 ± 1.9	5.5 ± 1.8	46.9 ± 3.1	9.6 ± 2.0
Japanese (20)	3.0 ± 0.5	21.5 ± 2.4	10.3 ± 2.5	3.0 ± 1.1	37.2 ± 2.3	14.8 ± 3.1
Koreans (21)	2.1	24.0	10.0	2.8	46.0	15.0
Chinese (23)	1.3 ± 0.2	16.3 ± 0.6	7.3 ± 1.0	3.9 ± 0.8	53.1 ± 2.0	14.7 ± 1.0
Americans (12)	2.0 ± 0.6	23.7 ± 0.7	5.8 ± 2.1	3.8 ± 1.3	53.2 ± 4.0	9.7 ± 2.5
Americans (12) PUFA diet	1.5 ± 0.96	20.2 ± 4.0	5.7 ± 2.3	3.7 ± 1.5	46.4 ± 5.1	18.9 ± 4.5

mortality in hypertensive Bahamians. Hypertension is often first documented when patients seek treatment for its complications.

Adipose tissue fatty acid composition has been determined in several populations. Some of the available data is summarized in Table V (12, 19–22). Linoleic acid (C18:2) content is most consistently high in Asian populations: Koreans at 15%, Chinese 14.7%, and Japanese 14.8%. The linoleate content of adipose tissue in Jamaican, West African, South African, and American groups is relatively low (5.8%–9.6%). Bahamian levels of linoleate approach those in the Asian groups. The highest linoleate levels reported, to our knowledge, are those of men following a diet high in polyunsaturated fats (12).

In a South African study, consistent relationships could not be found between adipose tissue composition, diet, and ischemic heart disease occurrence in white, Cape Colored, and Bantu subjects (21). In the United States, differences have not been found in the fatty acid composition of adipose tissue which distinguish subjects with and without ischemic heart disease (23). All of the ethnic groups listed in Table VI except Americans, appear to have a low incidence of coronary heart disease (CHD) although the methods used in assessing the presence of coronary heart disease were not standardized in the

TABLE VI

Adipose Tissue Linoleate and P/S Ratio in Living Subjects

	C18:2	P/S Ratio
New Yorkers (12)	9.7%	0.38
New Yorkers (12) High PUFA diet	18.9%	0.84
Japanese (20)	14.8%	0.76
Chinese (22)	14.7%	0.68
Koreans (13)	15.0%	0.53
Cretans (27)	10.4%	0.67
Bahamians	13.28%	0.51

various studies. On the basis of this data, there seems to be an inconstant relationship between the linoleate content of adipose tissue and the occurrence of ischemic heart disease, though populations with a P/S ratio of about 5 and above are characterized by low coronary heart disease prevalence.

Mortality statistics are not available on a regular basis from West Indian, African, and most Asian countries for comparative purposes. However, from a number of clinical and post mortem studies, it appears that in Haiti, Jamaica, St. Kitts Island, and in several African groups, heart disease develops infrequently despite a propensity to develop hypertension. While the relationships between dietary lipids, atherosclerosis and hypertension are complex, we submit the hypothesis that in the Bahama Islands, severe hypertension may not be associated with a high prevalence of coronary heart disease because dietary intake of unsaturated lipids, by exerting a serum cholesterol-lowering effect, negates, at least in part, the role of hypertension as a risk factor in coronary heart disease. A similar situation is suggested in Japan, although postwar changes in the Japanese diet, which progressively approximate American and European diet patterns, may eventually alter the present pattern of cardiovascular disease there (24).

If the relative percentage of depot linoleate reflects protection against the development of coronary heart disease in Bahamian subjects, it is pertinent to ask why in Cretan and Bantu populations, the relative linoleate is about 9 to 10% of total fatty acids—which is very similar to the linoleate observed in Americans. The answer to this question may lie in a comparison of the P/S ratio of the depot fats of these various populations and not in the sole consideration of their adipose tissue linoleate content.

The linoleate content of adipose tissue is compared to the P/S ratio in several populations in Table VI. The P/S ratio of the Japanese was 0.76, the Cretans were also relatively high—0.67, Chinese children had 0.68, Koreans 0.53, and Bahamians 0.51. All of these are considerably higher than the American level of 0.38. It is therefore observed that in no population group where coronary heart disease incidence is known to be low, is the P/S ratio less than 0.5. The highest P/S ratio, to our knowledge, is that of 0.84 found in New York men following a diet high in polyunsaturated fat for five years or more. We have focused on linoleic acid because its presence in adipose tissue is dependent on the dietary content of linoleate, since linoleic acid cannot be synthesized endogenously (6). In the Bahamas, the principal source of linoleate is probably fish, since lard and coconut oil are used commonly as cooking oils.

Published dietary analyses of several West Indian populations are compared to Japan and the United States in Table VII (24–27). All the West Indian groups ingest a lower percentage of dietary calories as fat (17–30%) compared to Americans (41%) (12, 22, 23). However, the West Indians consume more fat than the Japanese (24). In Haiti, about 27% of the dietary fat is linoleic acid (26). There are no comparable data for Nassau where, as in Haiti, 30% of dietary calories are estimated to come from fat. In Japan, linoleic acid constitutes 26% of dietary fatty acids. The principal sources of linoleate are vegetable foods, mainly soybeans (24).

TABLE VII

Dietary Patterns

Country	Calories	Protein	Fat	Carbohydrate
USA (28)	3,220	12%	41%	47%
Japan (26)	2,083	14%	13%	73%
Jamaica (28)	2,276	12%	17%	71%
Haiti (26)	1,572	11%	30%	60%
St. Kitts (25)	1,639	13%	17%	70%
The Bahamas	2,490	16%	30%	54%

Serum cholesterol levels of Bahamian subjects are not currently available. On the island of St. Kitts, however, 90% of 446 subjects studied had a serum cholesterol level below 200 mg% (25). Racial and ethnic differences and the disparate prevalence of hypertension in the Bahamas and St. Kitts (Table I), would suggest caution in comparing the two populations.

Summary

Preliminary studies suggest that in the indigenous Bahamian population, hypertensive cardiovascular disease is a prominent cause of morbidity and mortality; however, coronary heart disease is rarely diagnosed. On the basis of the high linoleic acid content in the adipose tissue of Bahamian children and adults compared to Americans, the hypothesis is proposed that in Bahamians, dietary sources of linoleic acid modify the effect of hypertension as a risk factor predisposing to coronary heart disease.

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Automation of Immune Hemolytic and Complement Reactions by AutoAnalyzer. II*

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This is Part II of a three-part work. Part I appeared in the previous (March–April) issue. Part III will appear in the July–August issue and will comprise Anticomplimentary Effects Automation of Complement Fixation Reactions, and the references for the entire manuscript.

The AutoAnalyzer is manufactured by Technicon Instruments Corp., Tarrytown, N.Y. 10591.

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ACKNOWLEDGEMENT
REFERENCES

Abbreviations and Symbols

A	Amboceptor—hemolysins, antibody against *E* which interacts with *C*
Ab	Antibody
AC	Anti-complementary substance
Ag	Antigen
C	Complement
C1, C2, ···	1st, 2nd, etc. components of complement
CH50 (CH100)	Units of complement yielding 50% (100%) hemolysis
CF	Complement fixation
CFR	Complement fixation reaction
D	Serum dilution
E	Erythrocyte = red blood cell
*E**	Erythrocyte damaged by complement
EA	Sensitized erythrocyte
EAC	Immune hemolysis due to interaction of E + A + C
GPS	Guinea Pig Serum
HD	Hemolytic Dose
MHC	Minimal hemolytic concentration
MHD	Minimal hemolytic dose
OD	Optical Density
R	Receptacle
RBC	Red blood cells
SRBC	Sheep erythrocytes
t	Time or duration of a reaction
T	Temperature usually C°
VBS 2+	Veronal buffer solution with added Ca^{2+} and Mg^{2+}

Abstract

This report is a summary of research and development involving complement fixation and immune hemolytic reactions evaluated by AutoAnalyzer. It includes description of adaptations by which this equipment can be used in serologic and serodiagnostic laboratories, experiments on temperature gradients, automatic serial dilutions, titration of complement components, the kinetics of complement fixation reactions, and the problem of anticomplementary sera.

4) HEMOLYTIC TITRATION OF COMPLEMENT AND ITS COMPONENTS

1. Titration of Guinea Pig Serum for Complement Fixation Reactions (CFR)

In this type of titration it is less important to know the absolute titer of *C* than to define the hemolytic dose (*HD*) needed for *CF*.

a. Three Automatic Titrations: Continuously Decreasing Dilution. Fig. 4-1 gives the flow diagram using *R*, exponential dilution, and 8 ml of 2% *GPS* placed in *R*. As shown in Fig. 4-1, the formula for dilution as a function of time is

$$C_t = 2\% e^{-0.1t} \qquad \text{Equation 4-1}$$

and the curve is sigmoidal.

Continuously Increasing Concentration. Titration is performed as above, but 4 ml of diluent is placed into *R* where it is continuously mixed with a constant addition of 2% *C*. The formula for this dilution is

$$C_t = 2\%(1 - e^{-0.2t}) \qquad \text{Equation 4-2}$$

as shown in Fig. 4-1 along with curve B.

Discontinuous Dilutions. The diagram of Fig. 4-1 is used with a sampler instead of *R*. Varying dilutions of *GPS* (2%, 1.8%, 1.6%, 0.6%, 0.4% and 0.2%) are placed in cups on the sampler where they are tested sequentially. A series of peaks is obtained as shown in *C* of Fig. 4-1.

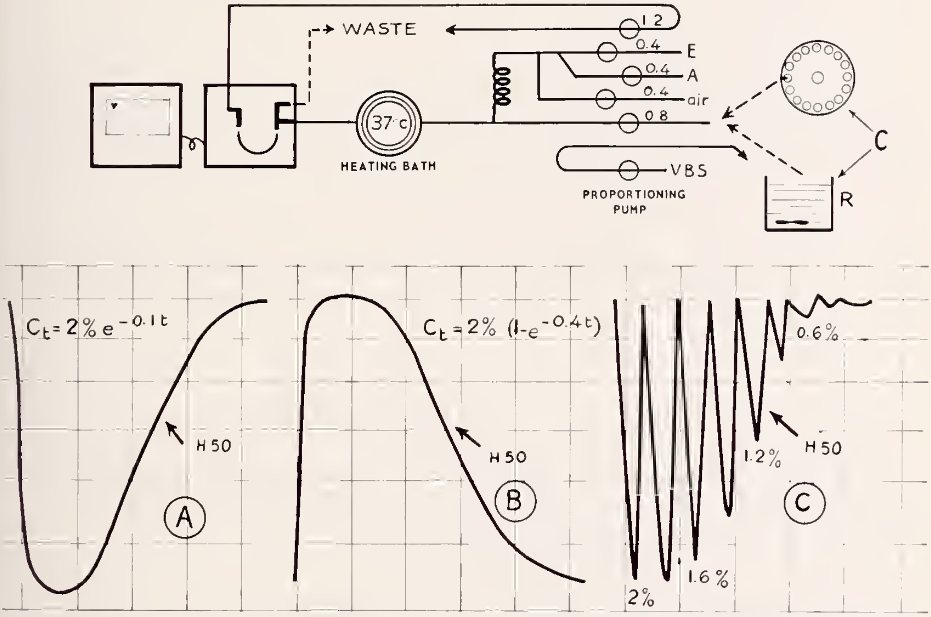

FIG. 4-1: *Titration of complement* (*GPS*). Top: Flow diagram; either of two techniques can be used, automatic dilution in R, or sampled static dilutions. Bottom: The curves are explained in text.

Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

TABLE 4-1

Comparison of % Hemolysis Achieved with Continuous and Discontinuous Dilutions of Complement (C)

Concentration:	2.0%	1.8%	1.6%	1.4%	1.2%	1.0%	0.8%	0.6%	0.4%	0.2%
Curve A	100	100	100	95	88	59	15	0	0	0
Curve B	100	90	80	55	15	5	0	0	0	0
Curve C	100	100	95	80	60	25	5	0	0	0

% Hemolysis:
Curve A: Continuous decreasing dilution of C.
Curve B: Continuous increasing concentration of C.
Curve C: Static dilutions of C.

Points in time of A and B were chosen to correspond to actual dilutions of C.

Analysis of Curves A, B, and C. Table 4-1 gives percent hemolysis from each curve in accordance with the concentrations of C assayed in curves A, B, and C. The three concentrations do not provide the same information. Results of curve C are between those of curves A and B. Curve A (decreasing concentration) gives results too high while curve B (increasing concentration) gives results too low. These differences are due to the "wash" problem inherent in continuous flow analysis. In moving from stronger to weaker concentrations, each sample is not diluted as efficiently as it should be, whereas the reverse takes place when the move is from weaker to stronger concentrations. This problem is reduced in the semi-automatic titration system in which each sample is separated from the preceding one by rinsing solution.

b. Von Krogh's Equation (42): In 1916, von Krogh proposed a formula to describe phenomena as different as protein precipitation by silver salts, bacterial agglutination, and hemolysis by alkali, cholera vibrio, and complement. It is an empirical formula today often referred to as logit-log transformation. The equation is

$$x = K\left(\frac{y}{1-y}\right)^{1/n} \qquad \text{Equation 4-3}$$

or

$$\log x = \log K + \frac{1}{n}\log\left(\frac{y}{1-y}\right) \qquad \text{Equation 4-4}$$

where x represents the quantity of fresh serum in ml, y represents the amount of hemolysis measured between 0.0 and 1.0, K is the constant which corresponds to the quantity of serum giving 50% hemolysis (since $x = K$ when $y = 0.5$); $1/n$ is an exponent expressing the slope of the line.

In summary, von Krogh's formula will linearize a sigmoidal curve. It has no theoretical significance, as von Krogh stated originally.

c. A Simple Linear Formula (43): A simpler formula will also express degree of hemolysis:

$$y = ax + b \qquad \text{Equation 4-5}$$

Here, y = degree of hemolysis, x = quantity of complement expressed in units of CH50, CH100, or *MHD*. Table 4-2 compares observed values with data calculated both by von Krogh's equation [$x = 1 \text{ unit CH50}(y/1 - y)^{1/5}$] and by this linear formula ($y = 2x - 1.5$). Data from linear calculations are closest to the observed data (Table 4-2).

TABLE 4-2

Comparison of Linear and von Krogh Equations with Experimental Data Regarding Degree of Lysis

Degree of Lysis (y)	CH50 Units of Complement		
	Experimental curve	von Krogh's equation	Linear calculation
0.10	0.78	0.65	0.80
0.20	0.85	0.76	0.85
0.30	0.89	0.84	0.90
0.40	0.96	0.92	0.95
0.50	1.00	1.00	1.00
0.60	1.05	1.08	1.05
0.70	1.10	1.18	1.10
0.80	1.17	1.32	1.15
0.90	1.24	1.39	1.20

From this equation we compute that

$$\left.\begin{array}{l}\text{1 CH100 unit is approximately 1.33 units CH50}\\ \text{1 CH50 unit is approximately 0.75 units CH100}\end{array}\right\} \qquad \text{Equation 4-6}$$

d. Instability of Diluted Complement: A correlation exists between dilution of *GPS* and spontaneous loss of hemolytic activity; the greater the dilution, the more rapid the loss. The technique of automation titration of Fig. 4-1 can be used to evaluate the rate of this loss. Measurements can be made continuously or discontinuously (using either *R* or sampler). In all cases a dilution of complement yielding 80% hemolysis is used to follow diminution of even minimal complement activity. Each experiment leads to an inactivation curve which is a first order reaction,

$$C_t = C_o e^{-kt} \qquad \text{Equation 4-7}$$

where k is the rate constant. The rate of spontaneous loss of 0.7% *GPS* depends on temperature and is given in Table 4-3.

Preservation of GPS Dilutions: It is advisable to prepare a stock dilution of 20% of reconstituted lyophilized *GPS*, preserve and refrigerate it at 4°C. Starting with a 20% dilution, other dilutions can be prepared at any time, but these should be made in cold *VBS* 2+ solution, and kept in an ice bath.

e. Daily Titer of Complement: Lyophilized lots of complement and available hemolytic sera are stable, and suspensions of *SRBC* are often relatively stable. When the three reagents *C*, *A* and *E* are stable, automatic apparatus provides doses which vary little from one day to the next. In automated serology, daily

TABLE 4-3

Effect of Temperature on Hemolytic Half-Life of 0.7% Guinea Pig Serum

Temperature	Time Constant (minutes^{-1})	Half-Life $t_{1/2}$
6°C	0.0003	40 hr.
20°C	0.0008	15 hr.
37°C	0.001	11 hr.
40°C	0.0012	10 hr.
42°C	0.005	2 hr. 15 min.
44°C	0.007	1 hr. 45 min.
47°C	0.025	30 min.

titrations of complement often can be replaced by a simple adjustment of incubation time to achieve immune lysis.

f. Adjustment of GPS Hemolytic Activity: MHD is a function of both *C* concentration and time of incubation of the *EAC* reaction at 37°C. For this purpose, the delay coils in which hemolysis takes place (flow time = 6 to 8 min.) are replaced by multiple glass mixing coils mounted in series as shown in Fig. 4-2. By increasing or decreasing the number of coils, the duration of hemolytic reaction time can be increased or decreased.

This series of delay coils has two advantages. (1) A fixed concentration of *GPS* and a minimum number of coils to obtain total lysis can be employed because the same conditions are useful for *CFR*. (2) In a long series of tests the lytic activity of complement frequently diminishes after several hours of work. This inconvenience can be overcome by increasing the reaction time for lysis to occur.

2. Titration of Complement in Clinical Medicine (44, 45)

The titration of hemolytic complement in the sera of patients as a measure of non-specific immunity was proposed by Wasserman in 1906. Although Wasserman's hypothesis was incorrect, the determination of complement titer remains a valuable test. Hypocomplemententemia is observed in glomerulonephritis, cirrhosis, prolonged septicemia, malaria, allergic disorders, dysproteinemias and systemic autoimmune disease such as lupus erythematosus. Hypercomplemententemia occurs in the acute phase of some diseases. Immunopathologic disorders

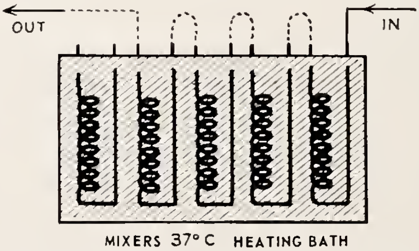

FIG. 4-2: *Battery of Delay Coils to Adjust Time for Hemolysis.* The duration of time to achieve hemolysis can be lengthened or shortened by adding or removing a delay coil.

as well as certain genetic deficiencies of complement components and/or inhibitors of complement components are associated with abnormal values.

a. Concepts of the Complement Unit: Mayer's Definition (*5*). "The hemolytic unit of complement, CH50, is defined as the amount in milliliters which will lyse 2.5×10^8 optimally sensitized red cells out of a total of 5×10^8 cells in the presence of optimal Ca^{++} and Mg^{++} at an ionic strength of 0.147 in one hour incubation at 37°C in a total volume of 7.5 ml."

Vargues and Audran's Definition (*46*). The serum complement titer is the reciprocal of the dilution of fresh serum which gives 50% lysis of red cells under the conditions used for immune hemolysis.

Example: It is necessary to dilute 4.0 ml of *GPS* 1/800 to lyse 50% of *SRBC* in a volume of 7.5 ml. The concentration of *GPS* is thus equal to $4/800 \times 7.5 = 1/1500$. According to Vargues, the titer of the serum is 1500 CH50 units. According to Mayer the titer is 200 CH50 units (i.e., 1500/7.5). The complement unit is, in any case, an arbitrary one. Its value depends on the concentration of *SRBC*, on their fragility, on the quality and quantity of hemolysins, on ionic forces, on pH, on the concentration of divalent cations, on temperature, and on duration of incubation. In addition, according to Mayer, the value depends on the final volume of the reaction. A precise measurement of complement requires rigorous control of titration conditions. The AutoAnalyzer assures this control.

b. Automatic Complement Titration: Table 4-4 summarizes three possible methods. In the three methods a certain number of parameters are fixed arbitrarily, namely: ionic strength (0.15), pH (7.3), diluent solution (*VBS* 2+), and quantity of *SRBC* (*EA*). Depending on the method, the variable will be either the concentration of *C*, the duration of time for 50% hemolysis (*t*H50), or the degree of hemolysis, *H*.

TABLE 4-4

Automatic Complement Titration

Methods	Complement (*C*)	Time (*t*)	Degree of Hemolysis (*H*)
Dose response	Variable	Constant (30–45 minutes)	Constant ($H = 50\%$)
Kinetic	Constant	Variable	Constant ($H = 50\%$)
Degree of hemolysis	Constant	Constant	Variable

Automatic "Dose Response" Titration. The flow diagram is represented in Fig. 4-3. The sampler is equipped with a special cam so that time of aspiration is 5 minutes and 30 seconds, rinsing time between samples is 30 seconds, and rate of sampling is 10 samples/hour. Each cup is filled with 2.4 ml of fresh human serum diluted 1:40. The sampler mixes each cup in sequence and, via two probes, both aspirates 0.8 ml serum dilution/min and introduces 0.4 ml diluent/min. Constant agitation insures a homogeneous mixture that becomes increasingly dilute in time. The constantly aspirated material is mixed with equal parts of *SRBC* and incubated at 37°C for 6 minutes. Transformation of time values (*t*) into concentra-

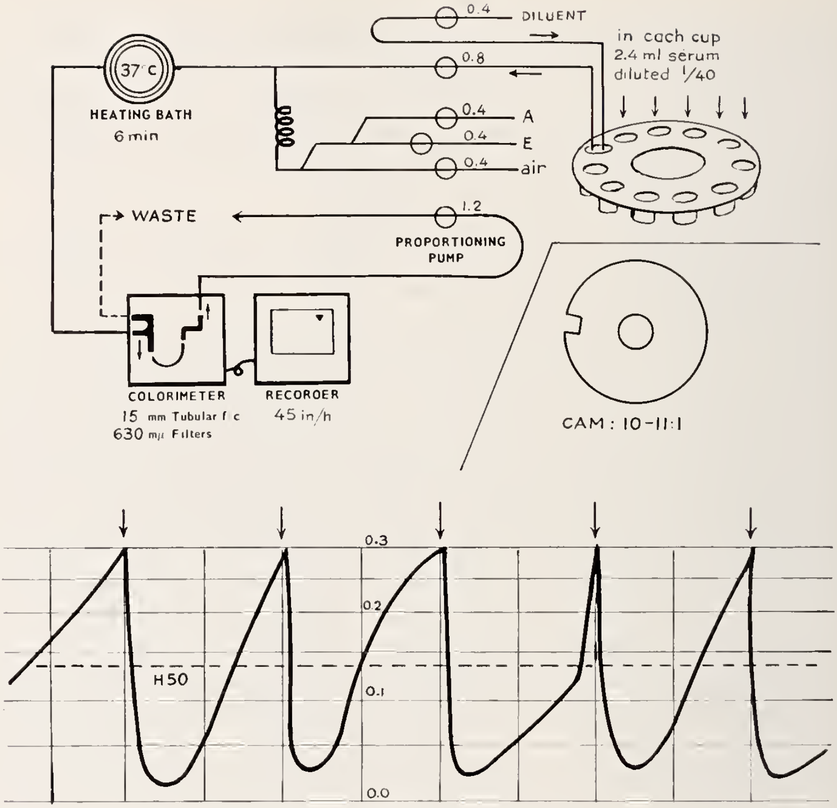
0.4
DILUENT
in each cup
2.4 ml serum
diluted 1/40
37°C
HEATING BATH
6 min
0.8
0.4
A
0.4
E
0.4
air
WASTE
1.2
PROPORTIONING
PUMP
COLORIMETER
15 mm Tubular f/c
630 mμ Filters
RECOROER
45 in/h
CAM: 10-11:1
0.3
0.2
H50
0.1
0.0

FIG. 4-3: *Continuous Linear Dilution.*
Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

tion (C_t) and CH50 units is made according to Table 4-5. The appearance of records is that of successive sigmoidal curves.

TABLE 4-5
Automatic Complement Titration

Time, minutes	0	1	2	3	4	5
Concentration, C_t	1/37	1/45	1/56	1/75	1/112	1/225
CH50 units ($1/C_t \times 40$)	150	180	225	300	450	900

Relation between time of continuous dilution of 1:40 fresh human serum, concentration, and CH50 units.

Semi-Automatic Kinetic Titration. The method consists of determining the time needed to lyse 50% of *EA*. The flow diagram is shown in Fig. 4-4. The sampler tray carrier is equipped with a 37°C water bath and the tray holds 9 ml cups. The timing cam is similar to that in Fig. 4-3. A stationary probe delivers *EA* into a cup placed three spaces before the cup actually sampled. When the apparatus is operating, cup *N* is in position for sampling. This cup contains 4.8 ml *EA*, and this suspension is aspirated as soon as it enters the aspirating position. At this moment, time zero, 0.1 ml of serum to be assayed is added and carefully mixed with the *EA*. Hemolysis then begins in this cup and is measured over a period of 5 minutes and 30 seconds. The probe then enters rinsing solution for 30 seconds. During 6 minutes cup ($N - 3$) will have received 2.4 ml of erythrocytes and 2.4 ml of diluted amboceptor, and during the next 6 minutes the contents of this cup will have been heated to 37°C. After sampling, the record provides a kinetic hemolytic curve for each cup of *EA* from which the time for 50% hemolysis (*t*H50) can be calculated; the number of CH50 units is derived from Table 3-1.

Semi-Automated Determinations of Degree of Hemolysis. This method performs 40 determinations per hour. The sampler, with a cam for 40 tests per hour and a 2:1 sample to wash ratio, uses 9 ml cups. Cup *N* faces the sampling probe, while a stationary probe delivers diluent (e.g., 5.1 ml in 90 seconds) into cup ($N - 3$). Each cup receives 0.1 ml of each human serum to be assayed, and the sampler stirrer maintains a homogeneous mixture of serum and diluent. Fig. 4-5 gives the flow diagram. Serum, diluted approximately 1/50, is sampled into the continuous flow of *EA*. The mixture is incubated through mixing coils, the length of which is chosen to provide 60% hemolysis with normal human sera. The recorder indicates a series of peaks. Hemolysis is greater than 60% if the serum contains excess hemolytic complement, and less than 60% if it contains too little. Table 4-6 lists the relation between degree of lysis and amount of lytic complement expressed in percentage relative to normal serum. Sera giving total lysis (greater than 125%

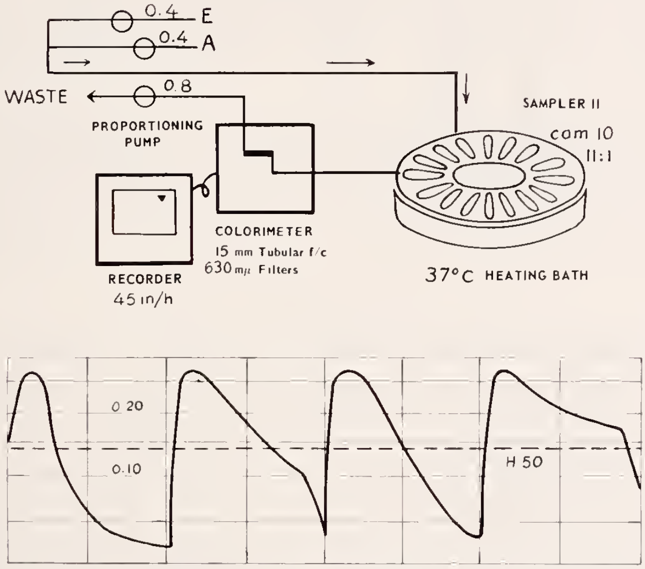

FIG. 4-4: *Complement Titration Achieved by Determining Time Required for 50% Hemolysis.*
Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

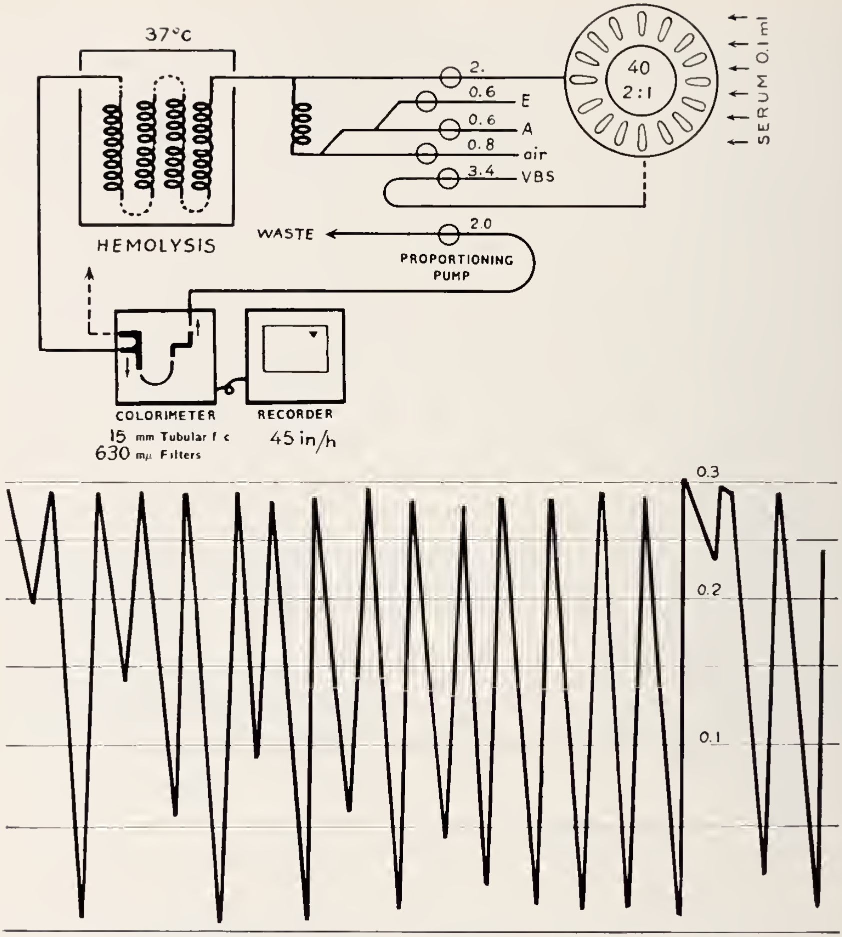
37°C
HEMOLYSIS
2.
0.6 E
0.6 A
0.8 air
3.4 VBS
40
2:1
SERUM 0.1 ml
WASTE
2.0
PROPORTIONING PUMP
COLORIMETER
15 mm Tubular f c
630 mμ Filters
RECORDER
45 in/h
0.3
0.2
0.1

FIG. 4-5: *Complement Titration by Determining Degree of Hemolysis.*
Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

of normal) and no lysis (less than 62% of normal) must be assayed more precisely.

TABLE 4-6

Relation between Degree of Observed Lysis and Relative Complement Titer

Hemolysis	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	0%
Complement titer	⩾125%	118%	112%	105%	100%	92%	85%	80%	74%	68%	⩽62%

These three methods of assaying hemolytic complement demonstrate the versatility of the AutoAnalyzer. The apparatus provides precise and reproducible values. The third method is best for rapid evaluations; the kinetic method is easy to use, and the first method should be used for reference work.

3. Automatic Assay of Components of Complement

Principle. Assay of components *C*1, *C*2, *C*3 and *C*4 formerly depended on *R* reagents, *R*1, *R*2, *R*3 and *R*4, but these reagents, although relatively easy to prepare, could not be standardized properly. The discovery of components *C*5, *C*6, *C*7, *C*8 and *C*9, and the possibility of preparing several components of complement as well as the intermediate cells *EAC*1, *EAC*4 and *EAC*1,4,2,3,5,6,7 have been associated with the evolution of new assay methods. Assay of a component now depends on the use of an excess of all components, except the one to be measured by virtue of its being the limiting factor of the lytic system. The commercial manufacturer of the reagents necessary for the titration of the components of complement (Cordis Laboratories, Miami, Florida) has produced standardized products. Automation of the assay of these components of complement poses few problems.

Reagents. Intermediate cells, *EAC*4, can be obtained from Cordis Laboratories or prepared as follows: a) Prepare *SRBC* in *VBS* 2+ at a concentration of 10^9 cells/ml. b) Sensitize 20 ml of this suspension, *E*, by adding 20 ml of amboceptor, *A*, diluted 1/300. c) Incubate at 37°C for 30 minutes to produce the *EA* complex and then cool to 0°C. d) Add 4 ml of *C*1 (Cordis Laboratories) diluted 1/30. e) Permit *EA* + *C*1 to incubate at 0°C for 20 minutes to form *EAC*1. f) Add 100 ml of C_{EDTA} (*GPS* + Na$_2$EDTA) and incubate for 15 minutes at 0°C. The complex *EAC*1,4 will form because *C*4 (but not *C*2) can be fixed in the absence of divalent cations. g) Two washings are performed at 0°C with *VBS*-EDTA solution which dissociates *EAC*1,4 into *EAC*4. h) Three washings are then made with *VBS* 2+ solution. The cells are finally suspended in 25 ml *VBS* 2+ solution, and concentration is adjusted to approximately 7×10^8 cells/ml. *EAC*4 can be preserved in a refrigerator for one week, but must be adjusted for use to a concentration of 10^8 cells/ml.

Automatic Determination of Value t_{max} for Titration of Components

Borsos has shown the importance of determining optimal incubation time of *EAC*1,4 with *C*2 to *EAC*1,4,2 because the latter intermediate complex dissociates spontaneously.

$$\begin{tikzcd}
EAC1{,}4 + C2 \arrow[r, dashed] & EAC1{,}4{,}2 \arrow[dl, bend left] \\
\text{decay} \arrow[u] \arrow[d] & \\
C2i &
\end{tikzcd}$$

Equation 4-8

The direct reaction is performed at speed, V_f, the decay reaction takes place at speed, V_d, and there exists an optimal time, $t_{\max}$, during which there is maximum formation of *EAC*1,4,2.

Fig. 4-6 indicates how the kinetics of *EAC*1,4,2 formation can be observed continuously. *EAC*4 is allowed to incubate for 20 minutes at 30°C with *C*1 to obtain *EAC*1,4, and 10 ml of this suspension is placed into *R*. When component *C*2 is

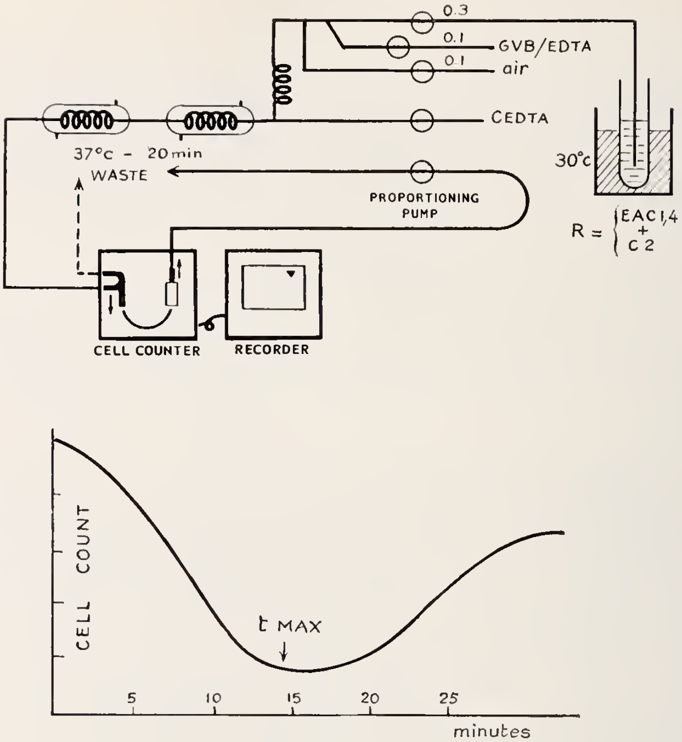

FIG. 4-6: *Determination of Optimal Time* (t_{max}) *for Formation of EAC1,4,2 from incubation of EAC1,4 with* C_2.
Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

added, the mixture is stirred carefully while R is maintained at 30°C and sampled continuously into C_{EDTA}. EDTA stops fixation of $C2$ but not of $C3$ and later acting components. After 20 minutes of mixing at 37°C, residual cells are determined by cell counter and a kinetic curve with U shape is recorded. From this record, the value of t_{max} can be determined.

Assay of Components C1 and C2. The flow diagram of Fig. 4-7 is employed. In the first stage the intermediate cells, $EAC4$, are transformed into $EAC1,4$ at 30°C. Then $C2$ is added to form $EAC1,4,2$ during optimal time, t_{max}. Thereafter, added EDTA blocks subsequent activity of components $C1$ and $C2$ before the final addition of C_{EDTA}. Terminal incubation takes place at 37°C. Non-lysed cells are determined by cell counter.

Assay of C1. Constant quantities of $EAC4$, $C2$, EDTA and C_{EDTA} are introduced but varied amounts of $C1$ are sampled to obtain a standard $C1$ curve. Thereafter, dilutions of serum in which $C1$ is to be assayed are sampled.

Kinetics of C1 Fixation. The same instrument used to assay $C1$ can be used to study $C1$ fixation. Ten ml of $C1$, diluted to correspond to one MHD, is placed into R. At time 0, an immune complex is added and fixation of $C1$ is followed.

Assay of C2. The flow diagram of Fig. 4-7 is used. Constant amounts of all

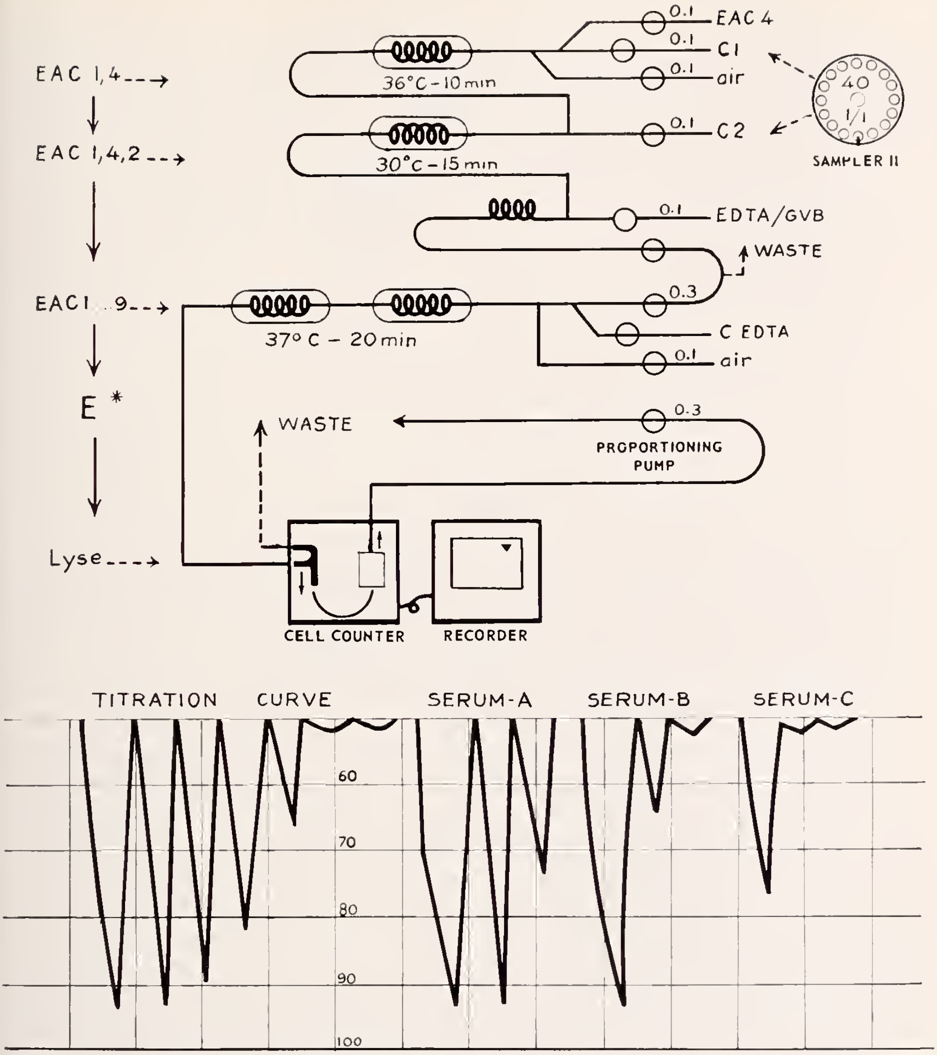
EAC 1,4
EAC 1,4,2
EAC1...9
E*
Lyse
36°C - 10 min
30°C - 15 min
37° C - 20 min
0.1 EAC 4
0.1 C1
0.1 air
0.1 C2
40
1/1
SAMPLER II
0.1 EDTA/GVB
WASTE
0.3
C EDTA
0.1 air
WASTE
0.3
PROPORTIONING PUMP
CELL COUNTER
RECORDER
TITRATION CURVE
SERUM-A
SERUM-B
SERUM-C
60
70
80
90
100

Fig. 4-7: *Titration of Complement Components C_1 and C_2.*
Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

reagents are used whereas *C2* is the limiting variable component. After establishing a standard *C2* curve, three different dilutions of unknown serum can be sampled to estimate dilution at 50% hemolysis.

Effective Molecules. Assay curves of *C*1 and *C*2 give the value of the dilution producing 50% hemolysis. The reciprocal of this dilution is the titer value for the *C*1 or *C*2 preparation. For estimation of effective molecules, Borsos (1, 47, 48) prefers 63% hemolysis rather than 50% hemolysis. The "one hit" theory postulates that only one site, *S**, is needed for cell lysis. If, for example, there are 100 effective *C*1 molecules and 100 *RBC* susceptible to lysis, the distribution of 100 *C*1 molecules among the 100 *RBC* obeys Poisson's law.

$$-\ln (1 - y) = m \qquad \text{Equation 4-9}$$

y represents the number of *RBC* having at least one S^* site, and m represents the mean of effective *C*1 molecules per *RBC*. The following equation is obtained in the above example:

$$-\ln (1 - y) = 1$$

or in $\log_{10}$ terms:

$$-\log (1 - y) = \frac{1}{2.3} = 0.43$$

$$\log (1 - y) = -0.43$$

hence

$$1 - y = 0.37 \quad \text{and} \quad y = 0.63 \qquad \text{Equation 4-10}$$

5) *KINETIC STUDIES OF COMPLEMENT FIXATION*

The kinetics of complement fixation by immune complexes have been studied by continuous flow analysis (31). The basis for such studies has been described by Osler (49, 50), Lundquist (51), Norden (52), and Vargues (53). At time zero, antigens, antibodies, and complement are mixed. Thereafter, samples are removed from the mixture for assay of residual hemolytic complement. The data indicate quantity of fixed complement as a function of time.

In Fig. 5-1, receptable, *R*, receives 10 ml *VBS* 2+ solution. At 30 second intervals thereafter, the following reagents are added in order: firstly, approximately 0.2 ml diluted guinea pig serum; secondly, a convenient quantity of antigen such as 0.1 ml; and thirdly, a convenient quantity of antiserum. At this point, the kinetic assay is at time zero and *R* is being sampled continuously. Material from *R* mixed with sensitized red blood cells, is incubated at 37°C in delay coils and turbidimetric evaluation finally performed by colorimeter. A typical curve is shown in Fig. 5-1.

In confirmation of earlier manual tests, complement fixation (*CF*) begins immediately, is most rapid in the beginning and diminishes progressively thereafter (37, 49–52). Complement fixation is a first order reaction comparable to virus neutralization (54).

1. *Model Curves*

Curves for complement fixation resemble curves for dilution of complement. If volume, V ml, of guinea pig serum corresponding to one CH100 unit is placed into Receptable, *R*, x ml of the contents can be removed per minute. At the same time, x ml diluent/min can be added, and the contents of *R* gently mixed by magnetic stirrer. Concentration of complement in *R* at time *t* is:

$$C_t = 1 \text{ CH100 unit } e^{-(x/V)t} \qquad \text{Equation 5-1}$$

When V is changed, the curve changes (Fig. 5-2), but every curve corresponds to

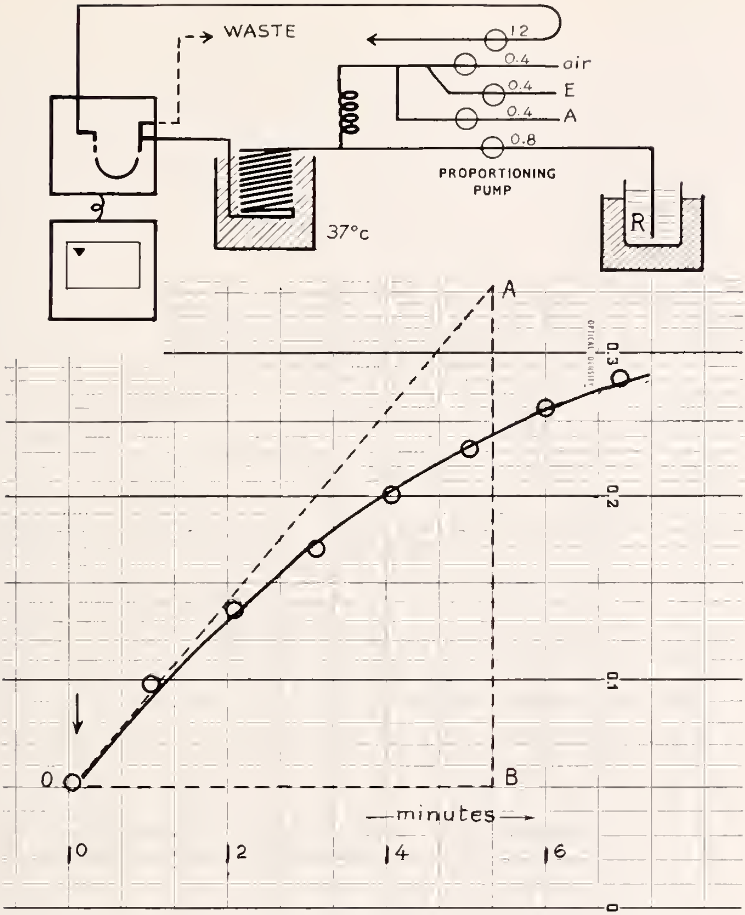

FIG. 5-1: *Kinetic Study of Complement Fixation by AutoAnalyzer.* The flow diagram illustrates the method: the phase of fixation occurs in the receptacle, R, and the duration of this phase is variable. The hemolytic phase occurs in delay coils immersed at 37°C for a fixed duration of time. The recorder furnishes a continuous curve which coincides with curves for exponential dilution of complement. The construction of triangle AOB gives the value of the tangent of the angle AOB.

equation 5-1. These curves are not truly exponential curves because they do not express C_t but only the degree of hemolysis corresponding to C_t. Thus, equation 5-1 is exponential, decreasing from 1 to zero, while curves increase from $OD = 0$ to $OD = 0.3$.

Curves of Fig. 5-2 were obtained with values of $x = 0.8$ ml/min, and values of V successively equal to 32, 16, 8, 4, and 2 ml. Each curve is characterized both by the value, x/V, and a tangential value at the origin. The value of the tangents is proportional to x/V. In effect, the exponential curve for equations of the following kind:

$$y = e^{-at} \qquad \text{Equation 5-2}$$

When

$$t = 0, \qquad \ln y = -a$$

With a true exponential curve, the value of the tangent at the origin is the value of a, if values of both abscissa and ordinate are of the same magnitude.

As far as the model curves of Fig. 5-2 are concerned, experiments showed a relation between values for (x/V) and tangents, as indicated by the following table:

TABLE 5-1

Values of V	32 ml	16 ml	8 ml	4 ml	2 ml
(x/V)	0.025	0.05	0.1	0.2	0.4
Measured tangents	0.3	0.6	1.2	2.5	5
Factor Tangent (x/V)	12	12	12	12.5	12.5

The Factor Tangent/(x/V) can be called the "constant of the machine" because the units for the abscissa and ordinate are of different magnitude. If they were the same, the constant would be the unit of the abscissa (1 minute) which measures ¾ inch or 18.5 mm. The unit of the ordinate should correspond to one unit CH100 if C_t were measured instead of hemolysis. If "zero hemolysis" corresponded to C_t and was approximately equal to 0.5 CH100 unit, the difference in length H between points 0% & 100% would be approximately 110 mm; thus, the CH100 unit of ordinate would be 220 mm, and the ratio size of unit of or-

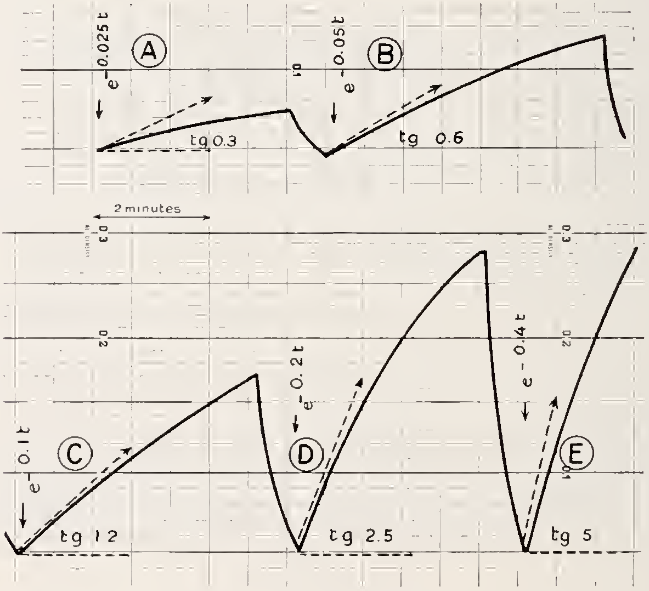

FIG. 5-2: *Model-Curves.*

dinate/size of unit of abscissa = 220/18.5 = 11.8 is close to being "the constant of the machine," experimentally found to be 12.

2. *Comparison between Model Curves and Kinetic Curves*

The following table compares the kinetic curve obtained by experiment and an exponential dilution curve:

TABLE 5-2

t (in minutes)	0	1	2	3	4	5	6	7
Kinetic curve	0.05	0.09	0.14	0.18	0.23	0.25	0.28	0.30
Model curve	0.05	0.10	0.14	0.17	0.22	0.24	0.27	0.29

The two curves are very close to one another. Consequently, calculations performed for one curve are useful for the other. To begin with the experimental curve (see Fig. 5-1) and a triangle, OAB, OB becomes 10 cm. The tangent equals AB/OB. For example, AB = 12.5 cm, the tangent = 1.25, the "constant of the machine" = 12. Therefore, for the constant of time, a,

$$a = \frac{\text{tangent at origin}}{\text{constant of machine}} = \frac{1.25}{12} = 0.1$$

The kinetic curve follows the equation

$$C_t = 1 \text{ CH100 unit} \cdot e^{-0.1t}$$

in which C_t represents free complement at time t. The quantity of fixed complement is given by the formula:

$$C_{\text{fixed}} = 1 \text{ CH100 unit } (1 - e^{-0.1t})$$

The value of $t_{1/2}$, or half-life, may be calculated from the formula

$$t_{1/2} = 0.69/a$$

From the numerical example of Fig. 5-1, $t_{1/2}$ would be equal to 0.69/0.1 = 6 min., 54 sec. This is the time needed for total inhibition of hemolysis, which confirms that 0.5 CH100 unit represents a threshold below which complement is ineffective. One could calculate the value of (a) by measuring the minimum time necessary for inhibition of hemolysis, 7 minutes in the example of Fig. 5-1, and dividing 0.69 by this time interval:

$$0.69/7 = 0.10 \text{ min}^{-1}.$$

Because kinetic curves of complement fixation resemble exponential dilution curves, they correspond to the following equations:

$$C_{\text{free}} \text{ at time } t = C_o \cdot e^{-at} \qquad \text{Equation 5-3}$$

and

$$C_{\text{fixed}} \text{ at time } t = C_o(1 - e^{-at}) \qquad \text{Equation 5-4}$$

where C_o represents the initial quantity of complement (close to 1 CH100 unit), (a) represents the constant of time in minutes^{-1}, and (t) represents time calculated in minutes.

3. The Kinetics of C1 Fixation

The kinetics of $C1$ fixation do not differ from what has been previously described. Experimentally, Fig. 4-7 is used. Receptacle, R, receives 10 ml *VBS* 2+ solution, then $C1$ corresponding to 1 CH100 unit, next antigen, and finally antibody. In essence, $C1$ is substituted for total complement (55).

The curves are exponential. The speed of fixation of the entire complement complex is comparable to the speed of fixation of the $C1$ component by the same immune complex. In Table 5-3, the antigen was a suspension of 10^8 Aeromonas/ml and antibody was serum of a hyperimmunized rabbit.

TABLE 5-3

	Fixation of Entire Complement Complex	Fixation of Component $C1$
Immunological system		
VBS 2+ solution	10 ml	10 ml
Guinea pig serum	0.2 ml	—
$C1$ component	—	0.2 ml
Antigen	0.2 ml	0.2 ml
Antibody diluted 1/10	0.2 ml	0.2 ml
Results		
Tangent at origin	1.5	2.0
Constant of machine	12	15
Time constant	1.2	1.3

Values for constants of time are identical because when guinea pig serum is used for total complement, $C1$ is no longer available for immune hemolysis as soon as it is fixed by the immune complex.

4. Kinetic Equations in C.F.

Virologists who study bacteriophage express the titer of antiphage sera by the rate constant of phage-antiphage interaction (64). Burnet (56) gave the first formulae for serologic kinetics in 1937. In 1943, Hershey (57), and in 1945, Delbruck (58) confirmed Burnet's formulae. First order kinetic equations were used by Tsuji (59), Talmage (60), and Hughes-Jones (61–62) in various aspects of serology. Immunochemists, Day (63), Froese (64), Tengerdy (65), and Dandliker (66) utilized second order equations. Results obtained with the AutoAnalyzer follow formulae 5-3 and 5-4.

Complement is a system formed of 9 different components. All chemical reactions involving more than two reactants progress by successive stages. At first, an antigenic structural grouping, Ag, combines with a specific antibody combining site, Ab, to form an elementary immune complex, $AgAb$:

$$Ag + Ab \rightleftharpoons AgAb \qquad \text{Equation 5-5}$$

This reaction is reversible. When antigen and antibody molecules are plurivalent, which is the usual situation

$$mAg + nAb \rightleftharpoons Ag^mAb^n \qquad \text{Equation 5-6}$$

If m is big enough, the product Ag^mAb^n becomes a precipitate.

The immune complex, $AgAb$, may fix complement, at least the first component, $C1$

$$AgAb + C1 \rightarrow AgAbC1 \qquad \text{Equation 5-7}$$

After the fixation of $C1$, the following successive stages are produced:

$$\begin{gathered} AgAbC1 + C4 \rightarrow AgAbC1,4 \\ AgAbC1,4 + C2 \rightarrow AgAbC1,4,2 \\ AgAbC1,4,2 + C3 \rightarrow AgAbC1,4,2,3 \qquad \text{Equation 5-8} \\ AgAbC1,4,2,3 + C5,\ C6,\ C7 \rightarrow AgAbC1,4,2,3,5,6,7 \\ AgAbC1,4,2,3,5,6,7 + C8,\ C9 \rightarrow AgAbC1,4,2,3,5,6,7,8,9 \end{gathered}$$

Equations 5-8 have been studied best with immune hemolysis (35, 67, 68) but the same sequence is followed in complement fixation.

Although complement fixation (CF) is an intricate process, experimental data furnish simple results which obey first order reaction laws (Equations 5-3 and 5-4). The kinetic curves of complement fixation do not show latency. In the kinetics of immune hemolysis, latency corresponds to the time necessary for all sequential reactions to take place. This phase is not apparent in CF and, apparently, only fixation of $C1$ is necessary to inhibit subsequent immune hemolysis. Equations 5-8, therefore, need not be considered because the kinetics of $C1$ fixation override those of subsequent reactions. For $C1$ to fix on sites of the Fc fragment of antibody, Ab, it is necessary and sufficient that Fc be modified after combination between Fab' and antigen (allosteric fixation). For IgG antibody, two antibody molecules need to be bound to the same antigenic particle; for IgM antibody only one molecule is needed.

Equations 5-6 and 5-8 can be written in kinetic form:

$$Ag + Ab \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AgAb + C1 \overset{k_3}{\rightleftharpoons} AgAbC1 \qquad \text{Equation 5-9}$$

where k_1 represents the rate constant of binding, k_2 represents the rate constant of dissociation, and k_3 represents the rate constant of $C1$ fixation.

Since the antigenic site, Ag, combines with an antibody site, Ab, the binding reaction is bimolecular. Through mass action, the rate of this reaction is:

$$\frac{dAg}{dt} = \frac{dAb}{dt} = -K_1(Ag)(Ab) \qquad \text{Equation 5-10}$$

The dissociation equation is monomolecular:

$$\frac{d(AgAb)}{dt} = -k_2(AgAb) \qquad \text{Equation 5-11}$$

These equations tend toward an equilibrium attained when both rates are equal:

$$k_1[(Ag)(Ab)] = k_2(AgAb) \qquad \text{Equation 5-12}$$

or the association at equilibrium =

$$K = \frac{k_1}{k_2} = \frac{(AgAb)}{(Ag)(Ab)} \qquad \text{Equation 5-13}$$

According to Day (63), binding of antibody by antigen is very rapid. It is certainly much more rapid than fixation of $C1$. The rate of $C1$ fixation by $AgAb$ is:

$$\frac{d(C1)}{dt} = -k_3(AgAb)(C1) \qquad \text{Equation 5-14}$$

In this equation $(AgAb)$ can be considered as very large in respect to $C1$ when little complement is used. $AgAb$ varies little during fixation of $C1$ so that Equation 5-14 becomes:

$$\text{rate} = -k(C1) \qquad \text{Equation 5-15}$$

where k represents the apparent rate constant of complement fixation. Equation 5-15 appears to be that of a pseudo-mono-molecular reaction, or a second order reaction that has degenerated to a first order reaction.

Equation 5-15 also defines the rate of standard complement fixation. This can be written:

$$\text{rate} = \frac{dC}{dt} = kC \qquad \text{Equation 5-16}$$

If the variables are separated: $dC/C = -kdt$. Integrating from O to C: $dC/C = -kdt +$ constant or

$$\ln C = -kt + \text{constant}$$

When the constant, t, is equal to ln Co

$$\ln = -kt + \ln C_o$$

whence

$$\ln \frac{C}{C_o} = -kt$$

and finally

$$C = C_o \cdot e^{-kt} \qquad \text{Equation 5-17}$$

Equation 5-17 is similar to equation 5-3 found by experiment.

The rate of formation of immune complexes depends not only on the concentration of antigen and antibody, but on their relative proportions. Equation 5-17 is applied when quantities of antigen and antibody are equivalent. In an excess of antigen, complement fixation will not occur. Prior formation of immune complex modifies its capacity for complement fixation because in the aggregate, Ag^mAb^n, some immunoglobulins are situated in the center of the aggregate and cannot fix $C1$.

5. *Factors Affecting Value of Rate Constant*

a. Quantity of Antiserum: A series of kinetic experiments have been performed, all similar to each other, except for the quantity of antiserum. The final concentration of antiserum used was 1%, 0.5%, 0.25% and 0.125%. For each concentration a kinetic curve was obtained from which the time constant, a, was calculated.

TABLE 5-4

Concentration of immune serum	1%	0.5%	0.25%	0.125%
Time constant (min^{-1})	2.0	1.0	0.5	0.25

There is a linear relationship between the concentration of immune serum and time. One can therefore calculate the rate, k, for a theoretical concentration of 100% antiserum.

$$\text{Rate Constant} = k = \frac{\text{time constant}}{\text{concentration}} = \frac{a}{D}$$

where D is a dilution of the immune serum in the final volume of the complement fixation reaction. In the example cited in the above table, $k = 200$ minutes^{-1}.

One can therefore write:

$$C_t = C_o \cdot e^{-kDt} \qquad \text{Equation 5-18}$$

which is a more general form of Equation 5-17. The equation is similar to one utilized in viral serology.

b. Temperature and Complement Fixation Kinetics: The rate of all chemical reactions is increased with a rise in temperature provided that the elevation of temperature does not produce secondary changes such as deterioration of reactants. With the AutoAnalyzer, effects of temperature on the time constant, a can be studied with the diagram of Fig. 3. A series of kinetic experiments using identical quantities of antigen, antibody, and complement can be performed, varying only the temperature of the water bath from one experiment to the next.

Using cardiolipin as antigen and serum of a syphilitic patient, Table 5-5 gives the following results:

TABLE 5-5

Temperature (°C)	5°	10°	20°	25°	30°	35°	45°
Value of a (× 100)	1.2	1.6	3.1	4.3	6.0	8.2	25
Value of $1/T$ (× 10^4)	35.9	35.3	34.1	33.5	33.0	32.4	31.4
ln a	2.07	2.20	2.49	2.63	2.77	2.91	1.39

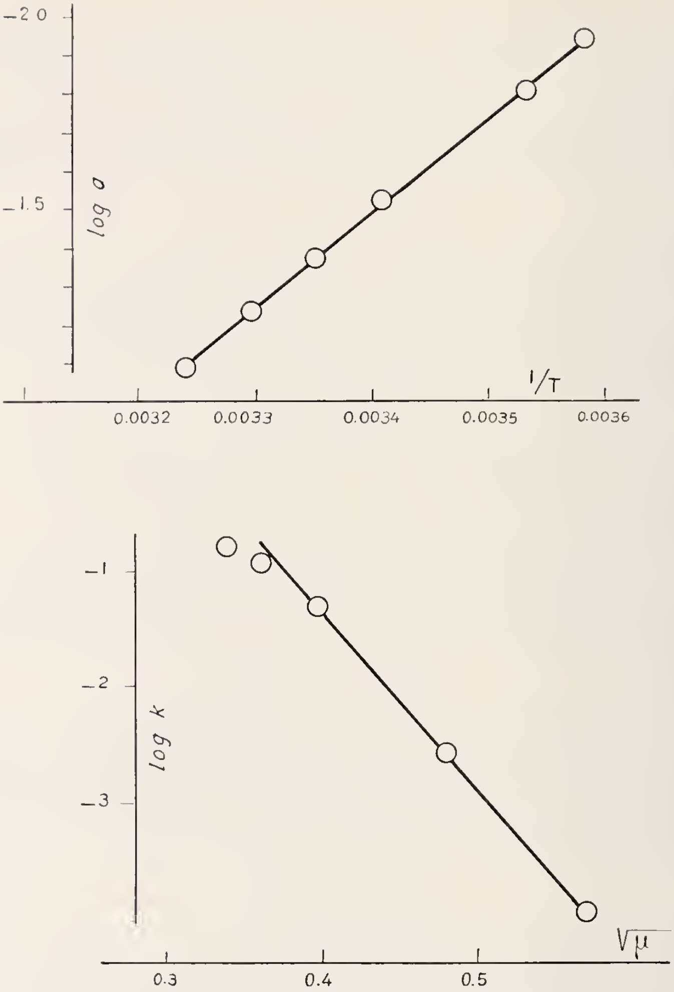

Fig. 5-3: *Effects of temperature (upper) and Ionic Force (lower) on the Speed of Complement Fixation.* The graphs of these figures correspond to the numerical examples given in the text.

The rate constant, *a*, increases with temperature. Kinetically, optimal temperature is that at which the greatest quantity of complement can be fixed in a given interval of time. An optimal temperature cannot be defined without knowing the duration of complement fixation. Time and temperature cannot be dissociated (69).

Constants which define the effect of temperature and the energy of activation *E*, can be calculated. The former is the coefficient of temperature Q_{10}. The rate of constants measured at two temperatures differing by 10°C is expressed by the ratio:

$$Q_{10} = \frac{k_T + 10}{k_T} \qquad \text{Equation 5-19}$$

In the numerical example given above, the coefficient of temperature between 30°C and 20°C is 6.03/3.1 = 1.93.

If the difference between T_1 and T_2 is not equal to 10°C, it is written:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{(10/T_2-T_1)}$$

or Equation 5-20

$$k_1 = k_2 \cdot Q_{10}{}^{(T_1-T_2)/10}$$

Most often, the value Q_{10} is close to 2. It is, however, only approximate. Q_{10} varies with temperature and is not the same between 10°C and 20°C as between 30°C and 40°C.

Arrhenius established a relation between rate constant, k, and absolute temperature, T.

$$\ln \frac{k_1}{k_2} = \frac{E}{R} \cdot \left(\frac{1}{T_1} - \frac{1}{T_2}\right) \qquad \text{Equation 5-21}$$

where k_1 is the rate constant at temperature T_1, k_2 is the rate constant at temperature T_2, E is activation energy, where R is a constant for perfect gases. E has been calculated for different complement fixation reactions. Values of order E equaling 12,000 calories have been found (53).

c. Ionic Force and the Rate of Complement Fixation (45, 70–71): Using the diagram of Fig. 5-3, with some modifications, dilutions of the solution were varied from 5 g NaCl/liter to 20 g NaCl/liter. A supplementary pump line was used to restore normal ionic conditions for the phase of immune hemolysis. Table 5-6 illustrates the results obtained:

TABLE 5-6

Ionic Force (μ)	0.12	0.14	0.16	0.24	0.32
$\sqrt{\mu}$	0.34	0.37	0.40	0.49	0.57
Rate Constant (k)	0.15	0.13	0.06	0.0013	0.0002
log k	−0.83	−0.89	−1.23	−2.64	−3.70

If one plots the square root of ionic strength against the logarithm of the rate constant, a straight line with a negative slope is found. Fixation of complement follows the law of Bronsted:

$$\ln k = k_0 + 1.02 \cdot Z_A \cdot Z_B \cdot \mu \qquad \text{Equation 5-22}$$

where Z_A and Z_B represent the electrical charges of substances A and B. The negative slope indicates that A and B carry opposite charges. Therefore, electrostatic forces are involved in complement fixation reactions.

d. Normal and Pathologic Human Sera: Human serum affects the kinetics of complement fixation. The rate of complement fixation is slowed when inactivated human serum is added to a standard complement fixation reaction using guinea pig serum as the source of complement. The diminution is greater as more human serum is added and the effect is more pronounced when sera of patients with rheumatoid arthritis are substituted. Kinetic experiments confirm previously published data and give a measure of the inhibition of tested sera (72–73).

6) *BASIC EQUATIONS FOR COMPLEMENT FIXATION*

1. *Fundamental Equation for CFR*

Equation 5-19 gives the value of the rate constant, k:

$$\ln \frac{C_o}{C_t} = kDt$$

For $\log_{10}$ values, the equation is:

$$\log_{10} \frac{C_o}{C_t} = \frac{kDt}{2.3}$$

or

$$k = 2.3 \left(\frac{1}{t}\right) \cdot \left(\frac{1}{D}\right) \cdot \log_{10} \left(\frac{C_o}{C_t}\right) \qquad \text{Equation 6-1}$$

The value of k is measured for a given temperature, T. The rate constant at 37°C, k_{37}, can be calculated:

$$k_{37} = 2.3 \left(\frac{1}{t}\right)\left(\frac{1}{D}\right) \log_{10} \left(\frac{C_o}{C_t}\right) Q_{10}^{(37-T/10)} \qquad \text{Equation 6-2}$$

Equation 6-2 is considered basic for it includes all principal parameters of complement fixation.

a. *Time of Fixation*, t, is expressed in minutes or seconds.

b. *Dilution*, D, corresponds to the dilution of serum that leads to 50% hemolysis inhibition ($Ct = 1$ CH50 unit) or 100% hemolysis ($Ct = 0.5$ units CH100). D must be calculated with respect to the final volume used in the fixation reaction.

c. *Initial Concentration of Complement*, C_o, is fixed in advance: either 2 100% units in the Kolmer technique, 5 CH50 units in the Osler technique, or 1 minimal hemolytic dose in the automatic test.

d. *Final Concentration of Complement*, C_t, indicates the quantity of complement which is not fixed at t minutes of fixation. If the dilution results in 50% inhibition of hemolysis, C_t will be equal to 1 CH50 unit or 0.75 CH100 units. If the highest dilution leading to total inhibition of hemolysis is the limit, C_t will be equal to 0.66 CH50 units or 0.5 CH100 units.

e. *Ratio* C_o/C_t is a pure number since C_o and C_t are expressed in the same system of units, either CH50 units, or CH100 units, or *MHD*. Therefore, the system of units chosen does not affect Equation 6-2.

f. *Coefficient of temperature*, Q_{10}, is equal to about 2, an approximation suitable for most calculations.

g. *Temperature*, T, is distinct from t, which represents time. In Equation 6-2,

T is expressed in degrees centigrade. Difference from 37, and the denominator, 10, are also in degrees centigrade so that T is a pure number.

h. *Constant*, k_{37}, is a value of an inverse ratio with respect to time (minutes −1) for D at 37°C (74).

Example: A complement fixation reaction has been performed with 0.1 ml of diluted antibody containing serum, 0.25 ml of antigen preparation, and 0.25 ml *GPS* (2 CH100 units). The mixture was incubated at 22°C for 60 minutes after which 0.3 ml of sensitized erythrocytes was added. The hemolytic phase lasted 30 minutes at 37°C. The diluted antibody containing serum at 1/50 gave 50% inhibition of hemolysis. From these data k_{37} is calculated as follows: $t = 60$ minutes. The true dilution, D, is $1/50 \times (0.1/0.6) = 1/300$ in the final reaction volume. The initial quantity of complement was $C_o = 2$ CH100 units and the final quantity of complement was $C_t = 0.75$ CH100 units, the quantity necessary for 50% hemolysis. Therefore the ratio $C_o/C_t = 2.66$, $\log_{10}$ of which is 0.424. Finally, temperature, T, is 22°C and $(37-22)/10 = 1.5$. Therefore,

$$k_{37} = 2.3 \cdot \frac{1}{60} \cdot \frac{300}{1} \cdot 0.424 \cdot 2^{1.5} = 13.8 \text{ min}^{-1}$$

The number, 13.8, expresses in an objective manner the complement fixing activity of the studied antibody containing serum.

The following table indicates how results are influenced by variations in serologic parameters.

TABLE 6-1

Serologic Parameters				Value of D	Sensitivity
t	C_o	C_t	T		
↗	↘	↗	↗	↘	↗
↘	↗	↘	↘	↗	↘

An antibody containing serum with activity corresponding to $k_{37} = 2.3 \text{ min}^{-1}$, can thus be analyzed by three different techniques, the parameters of which are indicated in Table 6-2:

TABLE 6-2

	Kolmer's Technique (4)	Osler's Technique (75)	Automatic Technique (76)
C_o	2 CH100 units	5 CH50 units	1 CH100 unit
C_t	100% inhibition (0.5 CH100 units)	50% inhibition (1 CH50 unit)	50% inhibition (0.75 CH100 units)
C_o/C_t	4.4	5	1.25
$\mathrm{Log}_{10} C_o/C_t$	0.643	0.698	0.130
t (minutes)	900	1200	40
T	6°C	4°C	37°C
$2^{\left(\frac{37-T}{10}\right)}$	8.5	9.7	1
A	0.014	0.013	0.0075

For Kolmer $D = 1/160$, for Osler $D = 1/175$, and for the automatic technique $D = 1/333$, which is twice as sensitive.

2. Time-Temperature Relation

As in enzymatic reactions, these two parameters are complementary in complement fixation. Complement fixation is better at 6°C than at 37°C only if the duration of fixation is 15 hours at 6°C and 30 minutes at 37°C (77).

Equation 6-2 defines the time-temperature relation. If a serum is characterized by a k_{37} constant and one wishes to obtain the dilution limit, D, in modifying time and temperature, the relationship existing between time of fixation, t, and temperature of fixation, T, is of the form:

$$t = A \cdot e^{-BT}$$

or Equation 6-3

$$\ln t = \ln A - BT$$

where

$$A = \frac{(\ln C_0 - \ln C_t)}{(k_{37})(D)} \text{ and } B = \frac{\ln Q_{10}}{10}$$

Equation 6-3 shows that t decreases in an exponential manner as T increases.

3. Optimal Temperature

Osler and Heidelberger (74) have shown that a larger quantity of complement is fixed at 6°C during 24 hours than at 37°C for one and ½ hours. In supposing a value of Q_{10} equal to 2, one can establish the following equivalences:

TABLE 6-3

Temperature	7°C	17°C	27°C	37°C
Duration of fixation	16 hours	8 hours	4 hours	2 hours

The lowest temperatures are effective only if the duration of fixation is very long (69). Experiments to determine optimal temperature of fixation will be described later.

4. Optimal Duration of Fixation

In all complement fixation reactions, two types of complement inactivation occur. One is specific fixation by the immune complex; the other is spontaneous deterioration of complement. Inactivation by CF is given by the formula:

$$C_t = C_o \cdot e^{-kt}$$

Spontaneous deterioration is given by the formula:

$$C'_t = C_o \cdot e^{-k't}$$

Specific fixation is indicated by the difference between C_t and C''_t. At time zero ($t = 0$), $C_t = C''_t = C_o$, and there is no difference. When t is very large, $C_t = C''_t = 0$, there is also no difference. Between these two values of t, the difference between C_t and C''_t increases and then decreases. The maximum may be calculated when the derivative of difference, $C_t - C''_t$, equals zero:

$$t\text{max} = \frac{1}{k - k'} \log_{10} \frac{k}{k'} \qquad \text{Equation 6-4}$$

This equation is of interest only if the value of k differs little from the value of k'.

The equation proposed for *CFR* is closely related to the one by Adams (54) for anti-phage sera:

$$k = 2.3 \cdot \frac{1}{t} \cdot \frac{1}{D} \cdot \log_{10} \frac{P_o}{P_t} \qquad \text{Equation 6-5}$$

NEUROLOGIC NOTES

JOHN LUSINS, M.D., EDITOR

Bilateral Homonymous Hemianopsia with Preservation of Central Vision

ALFRED T. FRONTERA, M.D.

Bilateral homonymous hemianopsia or cortical blindness is a relatively rare condition, but one that has been well documented in the literature. The most frequent cause of such a phenomenon is occlusion of the vessels supplying both occipital lobes. The literature, however, contains many reports of blindness due to ventriculography (1), vertebral angiography (2), trauma (3–6), shock (7), air embolism (7), and anoxia (7). The bilateral homonymous defects may be varied in the extent and portion of the field of vision implicated.

Bilateral homonymous hemianopsia with sparing of central vision (BHHS) is uncommon. In this paper two cases of BHHS are described and the visual and neurologic phenomena are discussed.

Case Report

Case 1. A 44-year-old, right-handed man was admitted January, 1958, to The Mount Sinai Hospital with neck and occipital pain, accompanied by nausea, vomiting, confusion, and inability to see "out of the sides of my eyes." There was no other associated neurologic symptomatology. The general physical examination was normal. The neurologic examination revealed no evidence of an organic mental syndrome or speech defect. There was a right homonymous hemianopsia to motion, form, and color using ten millimeter red objects with preservation of central vision for one or two degrees; there was a similar homonymous hemianopsia on the left with preservation of central vision at ten degrees (Fig. 1). Visual acuity was 20/25 O.D. and 20/20 O.S. The absolute size of the projected field increased with increased distance from the target. All eye movements on command were normal in all directions of gaze and on convergence. Optokinetic nystagmus using tape measure produced good pursuit with nystagmus opposite to tape movement in all directions of gaze. Ice-water calorics singularly and bilaterally were normal. The pupils were equal and reacted to light directly and consensually and to near vision. The flight of colors was normal. The remainder of the neurologic examination was normal. The skull roentgenogram, cerebrospinal fluid studies, electroencephalogram, right carotid angiogram, pneumoencephalogram, and left vertebral angiogram were normal. Evoked cortical potentials were normal. The patient was discharged with no change in neurologic status.

He was not reexamined until July, 1966, when he complained of nausea and vomiting accompanied by unsteadiness of gait and veering to the left. There was no change in his vision, no visual hallucinations, optic illusions, or diplopia. On examination, the visual findings were as previously described. There was now nystagmus on both lateral and upward gaze. There was a broad based, ataxic gait with staggering to the left. The Romberg was positive with falling to the left. Complete blood count, electrolytes, skull series, spinal fluid studies and left brachial angiogram were normal. A pneumoencephalogram and myeloencephalogram showed no ventricular filling of the posterior fossa and subarachnoid spaces.

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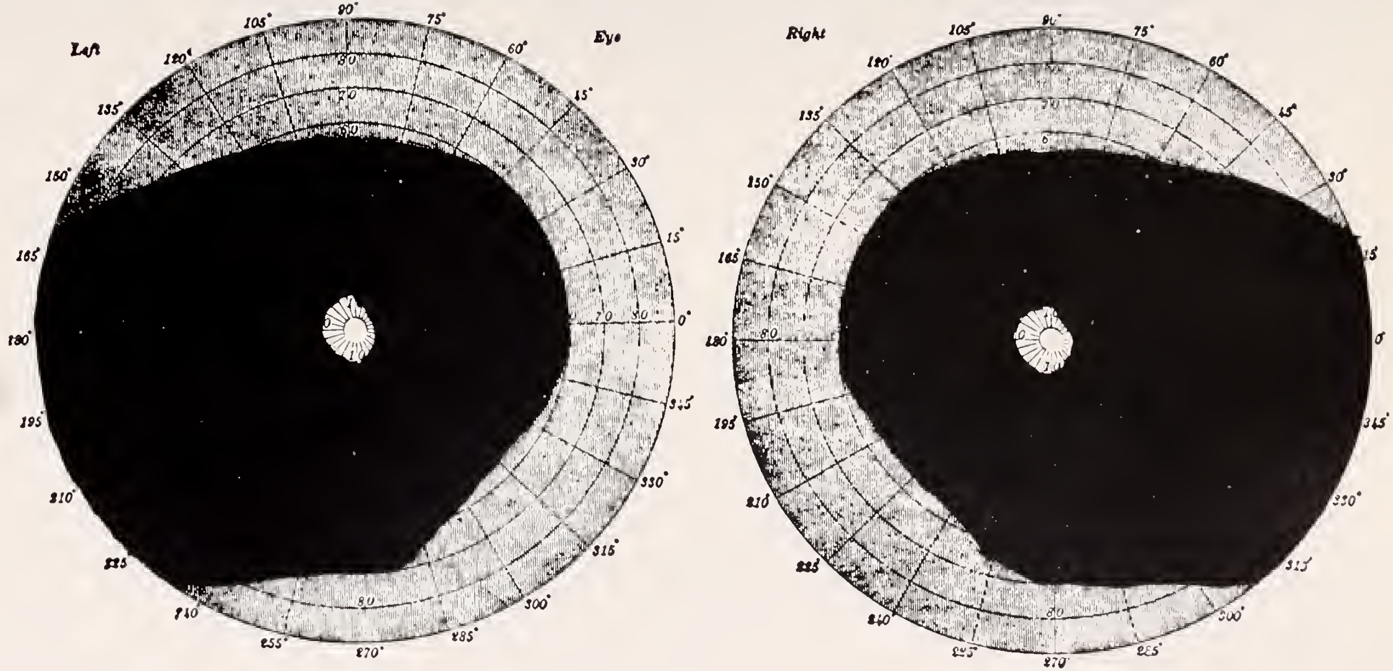
Left
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Fig. 1. A right homonymous hemianopia to motion, form, and color, using ten millimeter red object with preservation of central vision for one or two degrees; also, a similar homonymous hemianopia on the left with preservation of central vision at ten degrees.

Ventriculography showed no filling of the fourth ventricle or block of the aqueduct. Surgery revealed a right cerebellar hemangioblastoma which was removed.

Follow-up examination in 1970 revealed no change in visual findings.

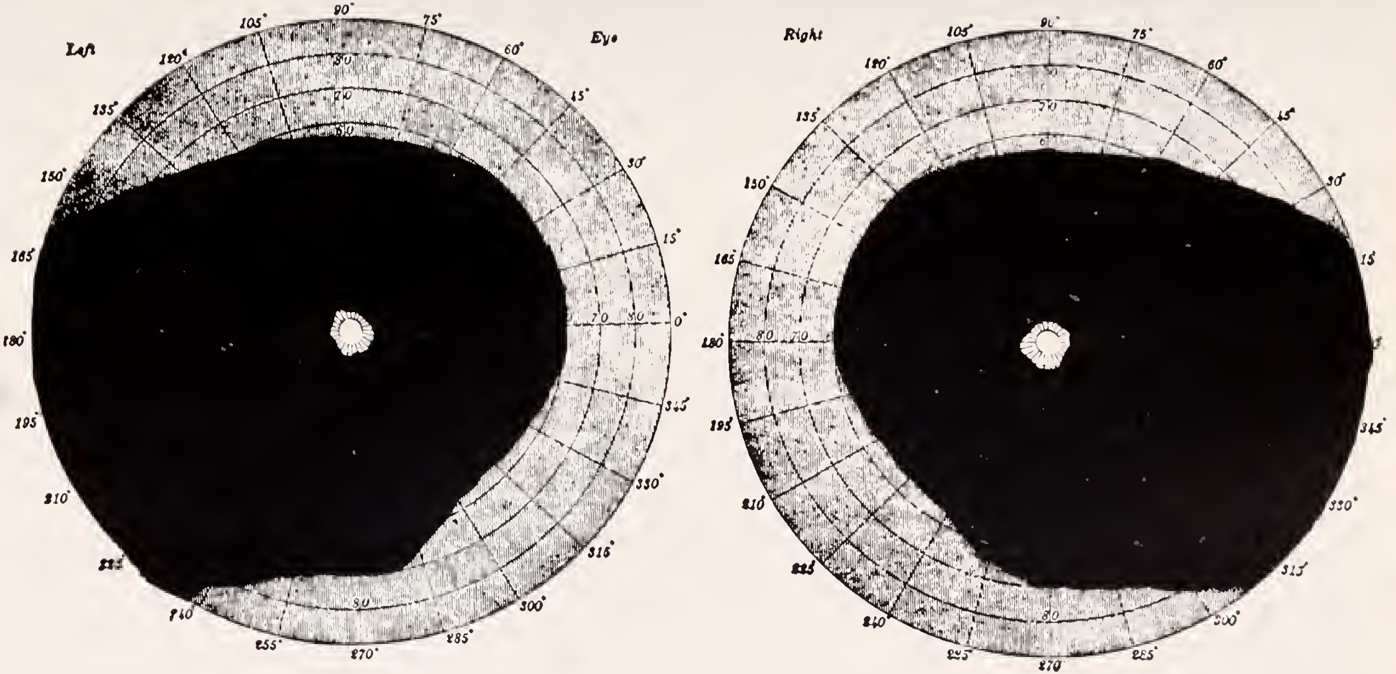
Case 2. A 71-year-old, right-handed man was admitted November 1971, because of "difficulty seeing." In September, 1971, the patient had a myocardial infarction and a period of hypotension lasting for about twenty-four hours after which he could not see clearly. He had difficulty recognizing faces and could see distant objects better than close ones. He had difficulty recognizing colors and was unable to read. There was no nausea, vomiting, vertigo, diplopia, numbness, weakness, or other neurologic symptomatology. General physical examination was normal. The neurologic examination revealed a mild organic mental syndrome. The visual acuity was 20/40 O.D. and 20/30 O.S. There was a left homonymous hemianopsia with preservation of color at one or two degrees, and a right homonymous hemianopsia with preserved central vision to three or four degrees. In the right hemimacular region there was some preservation of color vision to one to two degrees (Fig. 2). There was absence of visual after-sensation in the test of "flight of colors." Tests for pseudoisochromatic plates with either or both eyes were defective; the number twelve was read as two and the rest of the pseudoisochromatic plates could not be seen. The pupils were five millimeters in diameter and reacted to light. The fundi were normal. The extraocular movements were normal and there was no nystagmus. Optokinetic nystagmus and ice-water calorics were normal.

Complete blood count, electrolytes, chest roentgenogram, brain scan, EEG, and a spinal tap were normal. The EKG revealed a diaphragmatic infarction. A bilateral brachial angiogram showed poor filling of the posterior cerebral arteries.

A follow-up examination three months later showed improvement. The gross fields of vision disclosed ability to see motion at 60 degrees in the right temporal field, and in the peripheral portion of the left nasal field. There was a left homonymous hemianopsia to motion but with central sparing of vision; from the fixation point, he perceived movement to 3 degrees in the left homonymous field and 5 to 7 degrees in the right homonymous field. There was no perception of a three millimeter red target in the left homonymous central field. In the right homonymous field, the three millimeter red target was perceived at about 3 degrees to the right. The flight of color test was abnormal. There were no visual aftersensations except for one occasion when the patient saw green in the right eye. The pupils were equal and reacted to light and the fundi were normal. The eye movements were normal to command; there was no nystagmus. OKN was normal in the horizontal plane. There was difficulty in reading the pseudoisochromatic plates.

Discussion

Both of our cases of bilateral homonymous hemianopsia with central sparing exhibited many interesting phenomena. The contracted visual fields were jagged in outline, but homonymous in either half of the field of vision in each eye. They manifested so-called "funnel vision," i.e., there was enlargement of the visual field with increasing distance between the target and the patient. This is in keeping with the law of the visual angle which states that the visual angle equals arc tan S/D where S is the size of the target and D is the distance from the eye to the target. It is precisely through this law that one is able to perceive more of the surroundings as the distance between target and eye is increased. Thus, one may only be able to see a word or two when a target is held close to the face, but as the distance between the target and face is increased the whole target and, in fact, much of the background becomes visible. It is these two findings, the irregularity of the preserved area of vision and the preservation of the law of the visual angle, which help to distinguish between "tunnel or tubular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180°
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195°
345°
210°
330°
225°
315°
240°
300°
255°
285°
270°
80
70
50

Fig. 2. A right homonymous hemianopia with preservation of central vision at two degrees, and a left homonymous hemianopia with preservation of central vision at two degrees.

vision" and "funnel or peephole vision". The former visual fields are circular to outline and do not change with the variation in target distance; these are frequently hysterical in nature. The latter visual fields are usually found in disease of the occipital lobes.

Even though there was preservation of central vision and good visual acuity in both patients, each behaved as if blind. Both had trouble walking and finding their rooms, reading, and picking out large objects that were close to them. Such findings were pointed out by Terriem (8) and others and probably represent difficulty in recognizing large objects because of inability to see them as a whole secondary to the marked restriction of the visual fields.

Size constancy or form constancy was normal in both patients. A normal patient, through learning, is able to identify the size of a target in relation to the surroundings irrespective of distance between the eye and the target. In many cases of bilateral occipital lobe lesions form constancy has reportedly been lost and objects presented to patients may appear extremely small (micropsia) or relatively large (macropsia). Such phenomena were not manifested in our patients.

Case 2 exhibited an abnormal "flight of colors." In this test a bright light is held in front of each eye for ten seconds and the eye is then covered. In a normal subject there is positive after-imagery during which a series of colors—red, blue, green, yellow, etc.—appear over a period of thirty seconds to three minutes. These colors probably represent retinal cone elements which after excitation appear to react individually at different rates and thus cause the usual sensation of flight of colors. It appears that there is a high correlation between the presence of central vision and the presence of flight of colors (9). Thus, when any portion of the visual pathway mediating central vision is defective, the flight is defective. Case 1 had a normal flight of color while Case 2 had an abnormal flight of color.

Symonds showed that in several of his patients who had bilateral homonymous hemianopsia with sparing of central vision there was recovery of vision in a particular pattern (10). Bender, in studying the dynamics of hemianopsia, found that the visual defects began in the periphery of the field and proceeded centrally, and that in the recovery phase, the process was reversed (11). Such a phenomenon was exhibited in Case 2 but not in Case 1. In Case 2, the area of central vision increased and extended over a period of four months to include part of the peripheral field of vision.

The exact anatomical basis underlying these visual field defects is not entirely clear. The normal pupillary light reflexes show that the non-geniculate fibers of the optic tract to the pretectal areas were not damaged. The normal eye movements on command make it very probable that the fibers to the superior colliculi were intact. The angiographic evidence indicates that bilateral occipital lobe lesions accounted for the findings in these patterns.

It is known that central vision is represented in the posterior portion of the calcarine fissure. This posterior portion of cortex maybe supplied by anastomosis between the calcarine artery and the middle cerebral artery while the remainder of the striate cortex, i.e., that area involved in our cases, is supplied only by the calcarine artery, a branch of the posterior cerebral artery. It would seem, therefore, that in both our patients the cortex at the posterior portion of the calcarine fissure had been spared by the anastomotic blood supply though there was infarction of the medial portions of both occipital lobes.

The patients symptoms could be accounted for by either of three mechanisms: 1) occlusion of both posterior cerebral arteries proximally, 2) disease of the vertebral-basilar system, or 3) emboli arising proximal to the bifurcation of the basilar artery. In Case 2, there was a potential source of emboli from the recent myocardial infarction. Case 1 is more difficult to explain, but appears to be secondary to vascular disease of unknown etiology; definitive diagnosis awaits autopsy.

Acknowledgment

The author wishes to thank Dr. Morris B. Bender for the use of his cases.

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PEDIATRIC RADIOLOGIC NOTES

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CASE NO. 373

An infant was born with a large abdominal mass and anemia. An excretory urogram showed marked displacement of the right pelvocalyceal system inferiorly and medially. In addition there was distortion of the calyces indicating the intrarenal origin of the mass (Figs. 1A,B). The left kidney appeared normal.

At surgery, a large mass was found involving almost the entire kidney. The tumor had ruptured, probably during delivery, and there was free blood in the peritoneal cavity. A right nephrectomy was performed.

Pathologically the tumor was found to be a mesenchymal hamartoma. Ten months after surgery there has been no evidence of recurrence of tumor. The infant had been given one dose each of actinomycin D and radiation therapy, which were discontinued when the benign nature of the disease was evident pathologically.

Discussion

It has recently been recognized that the great majority of renal tumors presenting in the first weeks of life are benign mesenchymal hamartomas originating in the fetal kidney (1).

The radiologic features of this tumor are indistinguishable from Wilms' tumor. During the total body opacification phase of excretory urography, the mass may appear vascular, avascular, or mixed. Both tumors show calyceal distortion, indicative of the intrarenal location of the mass, and on angiography may show either hypervascularity or displacement of vessels around avascular areas.

Complete excision of the mesenchymal hamartoma is curative. Radiation therapy and chemotherapy are not only ineffective but potentially dangerous. Several deaths due to sepsis have occurred in infants with benign renal tumors treated with actinomycin D. Incomplete excision of the tumor may result in local recurrence which is treated surgically.

Preoperative radiation is often administered to older infants and children with intrarenal tumors presumed to be Wilms' before a tissue diagnosis is established. In the newborn, preoperative therapy is not indicated because the tumor is very likely to be benign.

The apparent better prognosis of Wilms' tumor in early infancy than in later

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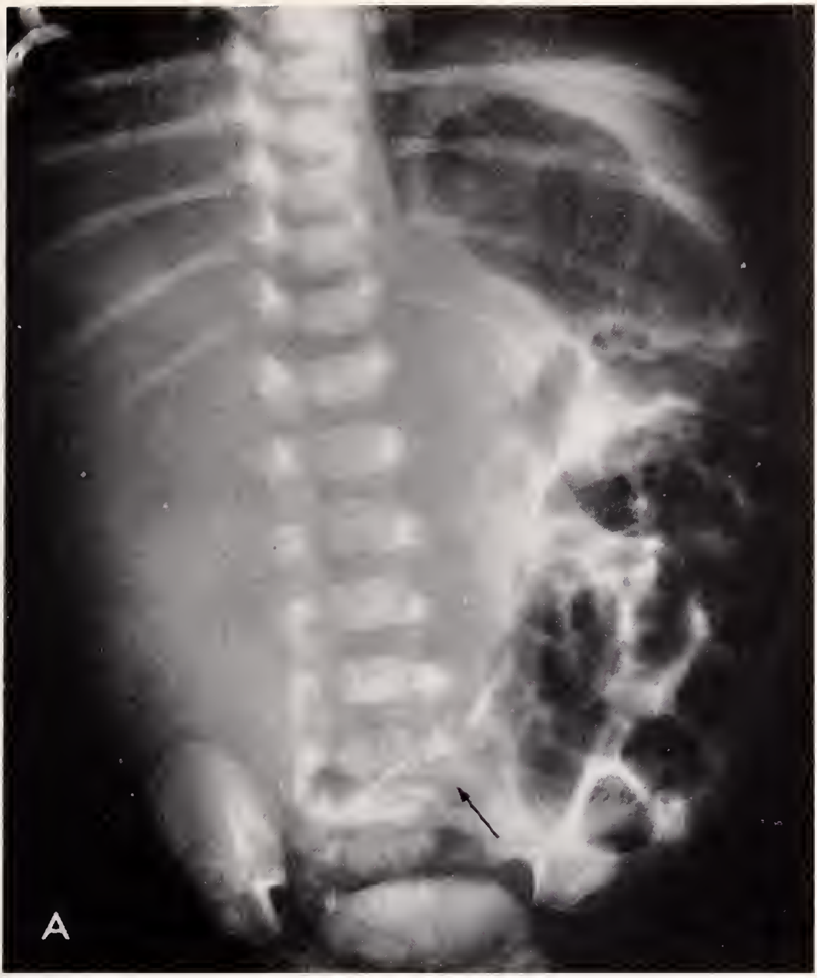

Case 373, Fig. 1A. Antero-posterior (slightly rotated) film from the excretory urogram shows a large, ill-defined mass occupying almost the entire right side of the abdomen. There is no bowel gas on the right. The right renal collecting system (arrow) is displaced inferiorly and to the left. There is distortion as well as displacement of the collecting system, indicating an intrarenal component to the mass. The left kidney, faintly seen, does not appear remarkable. Fig. 1B. Left posterior oblique film from excretory urogram confirms a normal left kidney and the displaced, distorted calyces of the right kidney (arrow) in the left lower abdomen.

childhood may be related to the inclusion of patients with mesenchymal hamartoma in the statistics, before it was recognized as a distinct pathologic entity. Synonymous with mesenchymal hamartoma are congenital mesoblastic nephroma of infancy, leiomyomatous hamartoma, and fetal renal hamartoma.

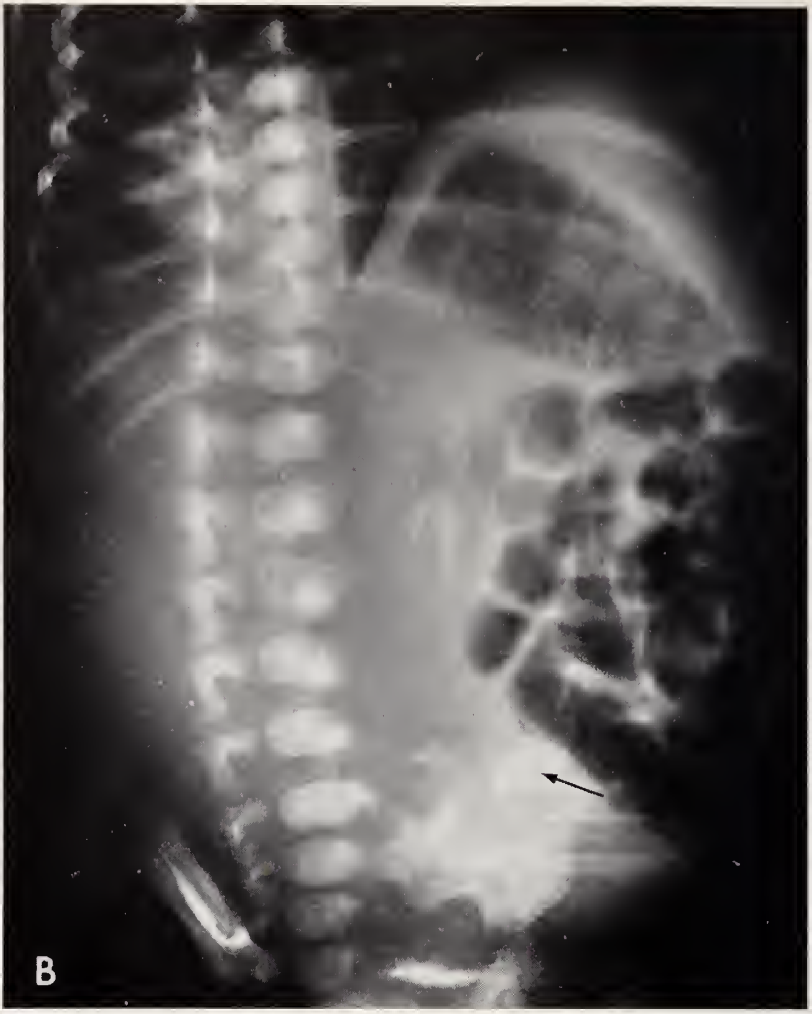

Fig. 1B

Case Report: MESENCHYMAL HAMARTOMA OF THE KIDNEY IN A NEONATE.

Reference

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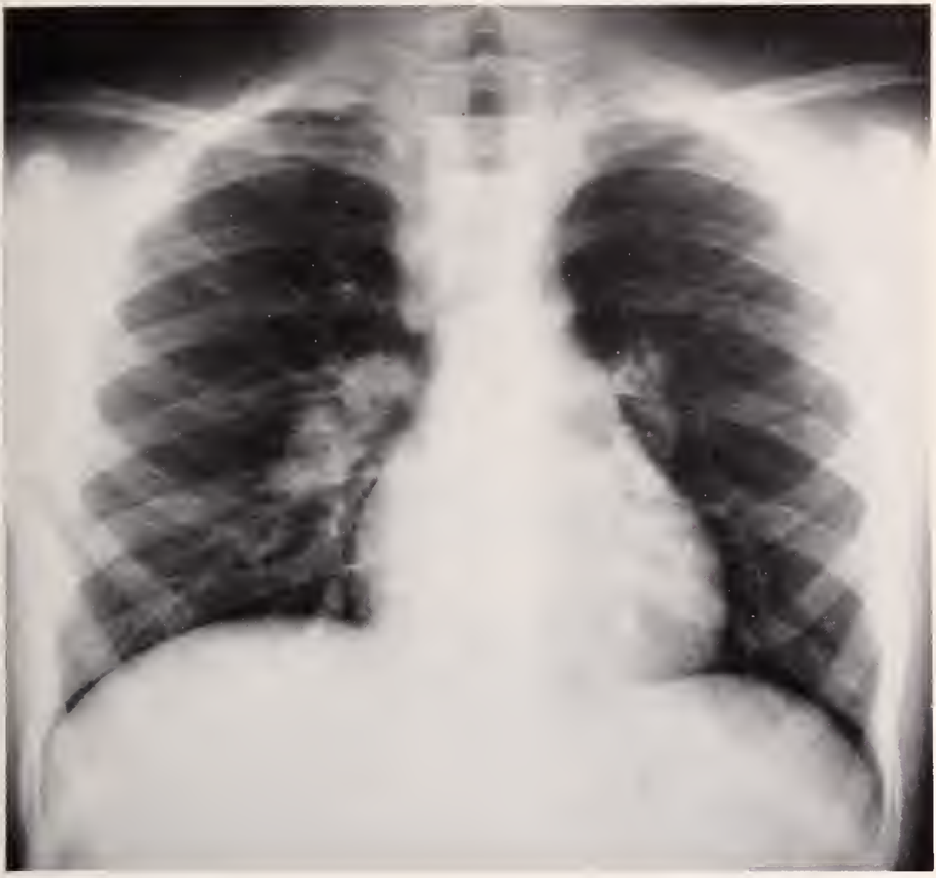
CASE NO. 374

A 7-year-old Caucasian girl was admitted to the hospital with a two-week history of fever, splenomegaly, and weight loss. The erythrocyte sedimentation rate was 84 mm per hour. Hemoglobin and white blood cell count were normal.



Case 374, Fig. 1. Postero-anterior view of the chest shows right hilar and paratracheal adenopathy. There is no evidence of involvement of the lung parenchyma.

There were 5% eosinophils in the peripheral blood smear. Skin tests for tuberculosis, atypical mycobacteria, candida, streptokinase, and streptodornase were negative. Complete ophthalmologic examination was normal.

The chest roentgenogram showed right hilar and paratracheal adenopathy (Fig. 1).

A scalene node biopsy showed noncaseating granuloma consistent with sarcoidosis.

Discussion

Sarcoidosis in children is generally thought of as a more severe and uncommon disease than in adults because it is usually first recognized in a symptomatic advanced stage with extrathoracic involvement. Asymptomatic adults with sarcoidosis are frequently detected in the course of routine or mass chest radiography. Exclusion of asymptomatic children from chest radiography may preclude the detection of the disease in its asymptomatic state and account for its apparent tendency to be rarer and more severe in children. There is evidence from Hungary and Japan, where mass chest radiography is carried out in children, that the prevalence rate of sarcoidosis is similar to that in adults (1).

Weight loss, cough, and fever are the most frequent symptoms and signs. The disease is found more frequently in Negro than Caucasian children and is unusual in children under nine years of age.

As in adults, the radiographic findings in the chest include bilateral hilar and right paratracheal adenopathy with or without involvement of the lung parenchyma (2). An atypical feature of this case is the unilaterality of the hilar adenopathy. This is a rare finding in adults, and its incidence in children is not known. Tuberculosis and lymphoma are to be considered in the differential diagnosis.

Acknowledgment

The editors wish to thank Dr. Howard Rappaport for permission to publish this case.

Case Report: SARCOIDOSIS WITH UNILATERAL HILAR ADENOPATHY IN A CHILD.

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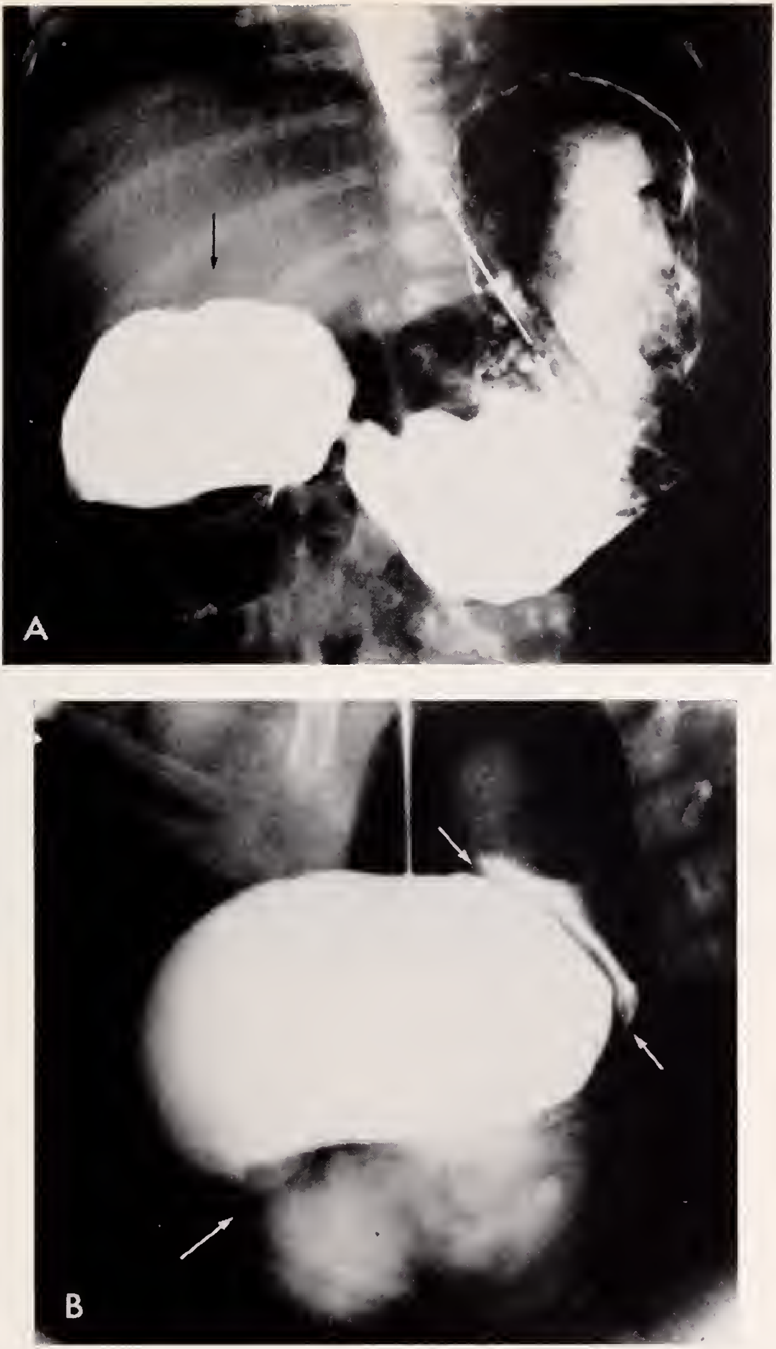
CASE NO. 375

A 1560-gram female was born to a 28-year-old primiparous woman after a 34-week gestation which had been complicated by polyhydramnios. The infant developed signs of high intestinal obstruction during the first day of life, with radiologic signs of proximal jejunal obstruction.

At operation, a complete diaphragm was found and resected in the proximal jejunum. The gastrointestinal tract was patent distal to the jejunal diaphragm. A gastrostomy was performed, and patency proximal to the jejunum was tested by passing a #5 infant feeding tube through the gastrostomy into the duodenum and proximal jejunum just distal to the resected diaphragm.

Soon after oral feedings were given, clinical and radiologic signs of partial duodenal obstruction appeared. A gastrointestinal series at seven weeks of age showed a very large proximal duodenum with no well-formed descending duodenum (Fig. 1A). A thin diaphragm was demonstrated obstructing the proximal duodenum. This was shown in profile in a steep right anterior oblique projection (Fig. 1B). The diaphragm caused marked obstruction when the patient was recumbent, but in the erect position duodenal emptying was facilitated. At three months of age, a duodenal diaphragm with a small central fenestration was visualized at surgery and a duodenojejunostomy was performed. The patient has done well postoperatively.



Case 375, Fig. 1A. Postero-anterior view of the stomach and duodenum shows marked dilatation of the bulb and proximal duodenum (arrow). The pylorus is patulous. Fig. 1B. Steep right anterior oblique view shows a sharply defined curvilinear lucency of the web (upper two arrows) in the posterior aspect of the greatly enlarged duodenum. Projected inferior to the duodenum is barium mixed with retained secretions in the stomach. The partially obscured pylorus (lower arrow) is displaced anteriorly by the large duodenum.

Discussion

When congenital obstruction is present at two different levels of the intestinal tract, the proximal obstruction usually becomes clinically obvious first, and the distal one may be overlooked unless the entire bowel distal to the proximal obstruction is checked for patency at the time of surgery. In this infant, the more distal obstruction presented first because it was complete, while the more proximal obstruction was partial. At the time of surgery for resection of the jejunal web, a more proximal obstruction was sought but not appreciated because of the fortuitous passage of a thin catheter through the central fenestration in the duodenal web.

It is now generally accepted that most intestinal atresias distal to the duodenum are the result of intraabdominal vascular accidents in the fetus (1). The duodenum is the only portion of the intestinal tract in which a fetal solid stage has been proved. Stenoses and atresias (including webs) of the duodenum are thought usually to be caused by a failure of recanalization of the epithelial core which temporarily occludes the duodenal lumen during the early weeks of fetal life. An atretic segment of either etiology may consist of a long epithelial plug or only a thin diaphragm which may be perforated in one or more places.

While the presence of duodenal obstruction was apparent radiologically, demonstration of the duodenal diaphragm required precise positioning of the patient. Rotation of only a few degrees from the ideal position allowed visualization of the large duodenum, but not of the diaphragm itself. The pylorus was also difficult to identify because of its marked anterior displacement by the enlarged obstructed duodenum (Fig. 1B). The lucent curvilinear outline of the internal duodenal diaphragm may mark the point of attachment to the duodenal wall or may be considerably distal to its actual origin, as in the "windsock" type of diaphragm.

Case Report: DUODENAL AND JEJUNAL DIAPHRAGMS IN A PREMATURE INFANT.

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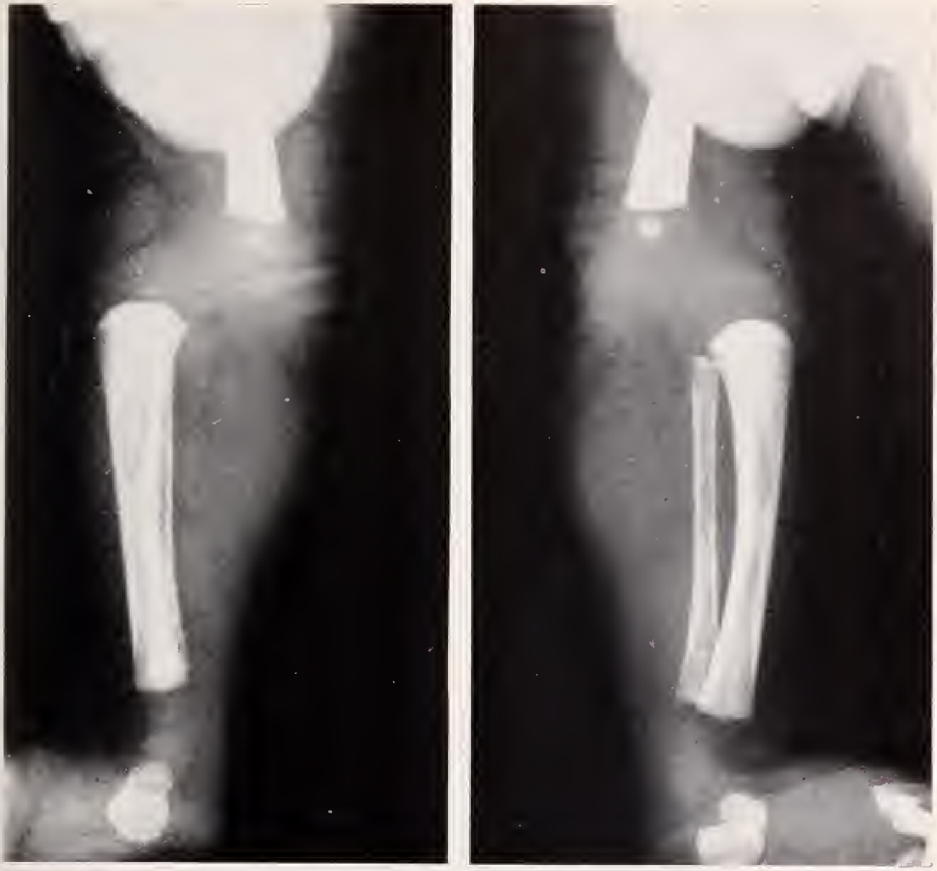
CASE NO. 376

A male infant was born with dislocations of multiple joints. Most striking were anterior dislocations of both knees (Fig. 1). In addition, the hips were completely dislocated (Fig. 2), and there was dislocation of the left proximal radius (Fig. 3). Cavus deformities were present in both feet. The metacarpophalangeal joints in both hands were dislocatable. There was generalized joint laxity. The eyes appeared widely spaced, and the root of the nose was broad. The palate was normally formed. Neurologic examination, electromyography, and conduction velocities were normal.

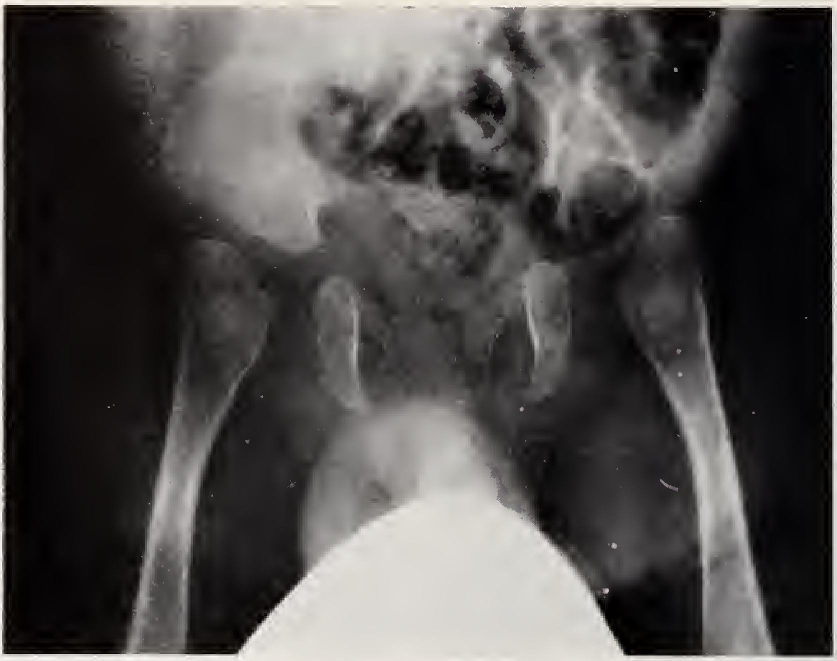
The mother was a 16-year-old primiparous girl who was congenitally deaf.



Case 376, Fig. 1. Lateral views of both knees show anterior dislocations of the tibiae and fibulae on the femora.

The pregnancy had been normal. A caesarean section was performed for cephalopelvic disproportion.

Radiologic examination demonstrated the obvious dislocations as well as numerous minor skeletal abnormalities. There was lack of normal tubulation of the distal tibiae, fibulae (Fig. 4), and distal radii and ulnae (Fig. 3). The distal tibial metaphyses were V-shaped rather than linear (Fig. 4). The distal radial epiphyseal ossification center was ossified at birth, although the capitate and hamate were not. (Normally the capitate and hamate ossify at three months and the distal radial epiphysis at 15 months in males.) The third and fourth metacarpals and middle phalanges of the fifth fingers were short. There was notching at the base of the distal phalanges of the thumbs (Fig. 5). Despite the clinically widely spaced eyes, there was no orbital hypertelorism radiologically (Fig. 6).



Case 376, Fig. 2. Antero-posterior view of the pelvis and hips shows gross dislocation of both hips superiorly and laterally.

Discussion

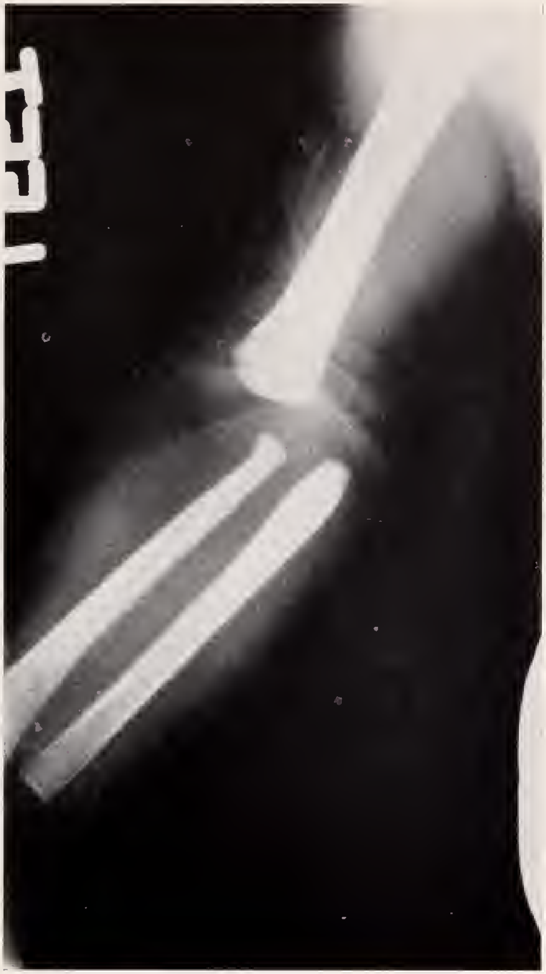
The essential requirements for the diagnosis of Larsen's syndrome are the combination of anterior knee dislocation and a characteristic facial appearance: a flat facies with apparently widely spaced eyes (1, 2). Additional findings which may lend support to the diagnosis, but are not invariably present, are cleft palate and dislocation of additional joints. A generalized chondro-osseous dysplasia is reflected in the frequent presence of accessory ossicles in many areas (hands, wrists, knees, elbows, feet) and deficiency in the pubis, spine, or phalanges.

In contrast to arthrogryposis multiplex congenita, the dislocations of Larsen's syndrome occur with flaccid rather than contracted joints. Degeneration of anterior horn cells is found in arthrogryposis, but there is no neurologic deficit in Larsen's syndrome.

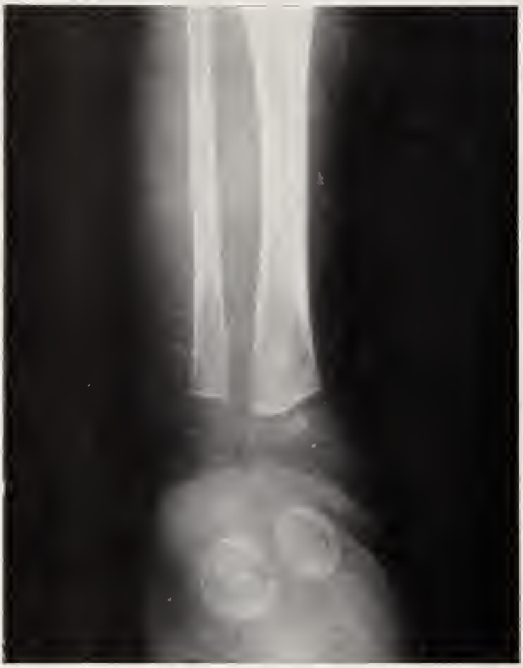
Although the characteristic facial appearance of Larsen's syndrome includes widely spaced eyes, the frequency of actual bony hypertelorism is not known.

The inheritance of this syndrome is variable. Both dominant and recessive forms may exist.

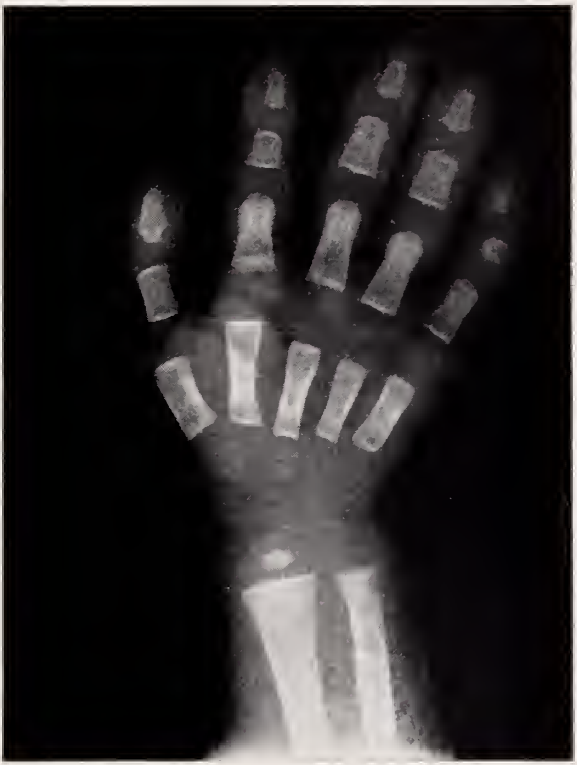
Case Report: LARSEN'S SYNDROME (MULTIPLE CONGENITAL DISLOCATIONS WITH CHARACTERISTIC FACIES).



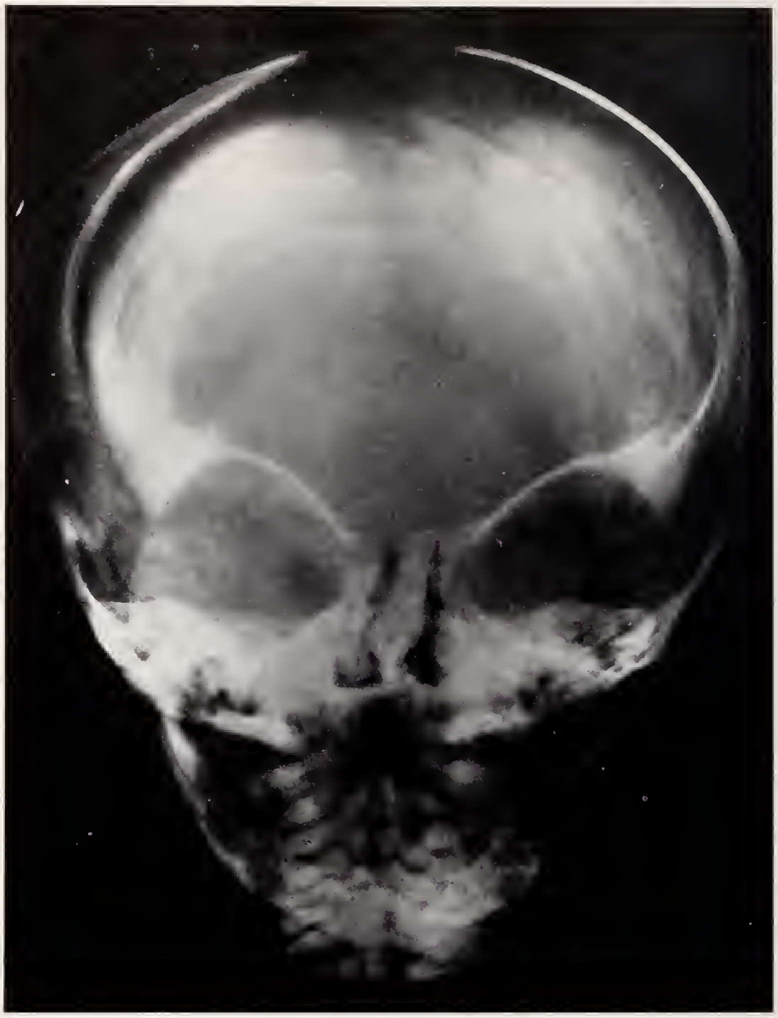
Case 376, Fig. 3. Antero-posterior view of the arm and forearm shows medial dislocation of the radius and ulna on the humerus and lack of modeling of the distal radius and ulna.



Case 376, Fig. 4. Antero-posterior view of the leg and ankle shows lack of normal tubulation of the distal tibia and fibula. The distal tibial metaphysis is V-shaped rather than linear.



Case 376, Fig. 5. Postero-anterior view of the hand and wrist in the newborn period shows ossification of the distal radial epiphysis (normal time cf appearance is 15 months). The third and fourth metacarpal and middle phalanx of the fifth finger are short. The base of the distal phalanx of the thumb is notched.



Case 376, Fig. 6. Antero-posterior view of the skull shows a normal interorbital distance. The large anterior fontanelle is normal for a newborn.

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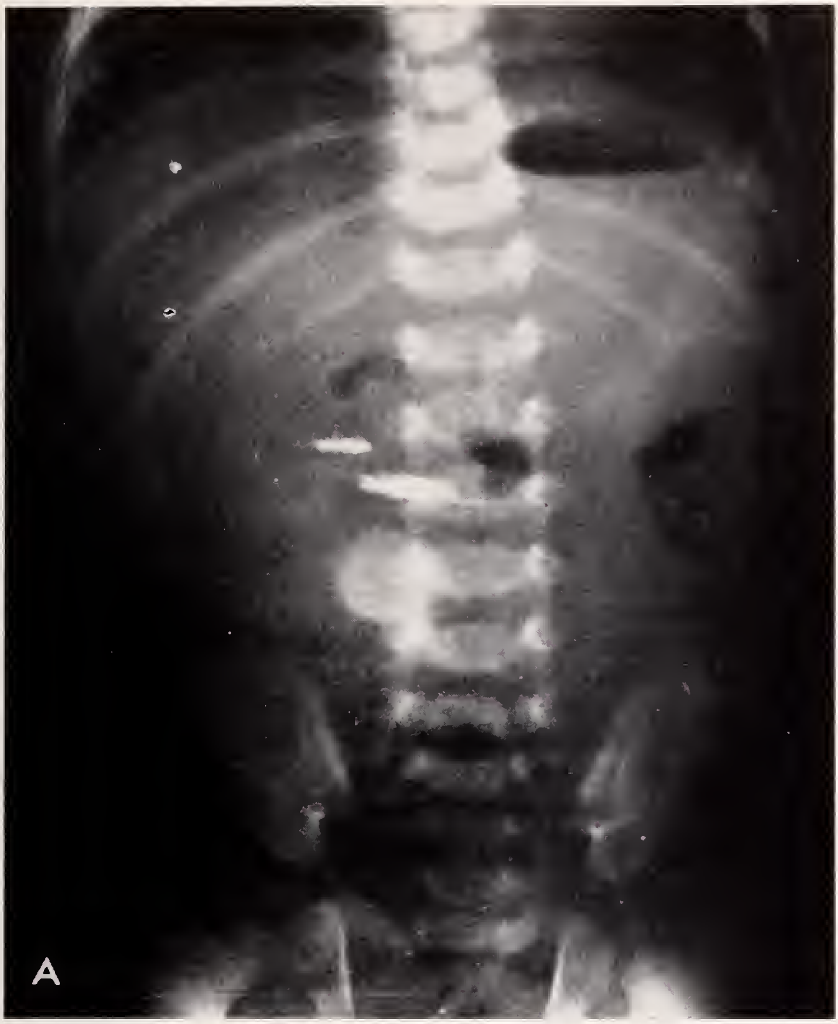
1. Silverman, F. N.: Larsen's Syndrome: Congenital Dislocations of the Knees and Other Joints, Distinctive Facies, and Frequently, Cleft Palate, Ann Radiol 15:297–328, 1972.
2. Bloch, C., and Peck, H. M.: Radiologic Notes. Case #251, Bilateral Congenital Dislocation of the Knees, J Mount Sinai Hosp 32:607–609, 1965.

CASE NO. 377

An eight-day-old male infant was admitted with a 12-hour history of bilious vomiting. He was the product of a normal pregnancy and delivery. At two days of age, he experienced three episodes of bright red blood in the stool. An Apt test



Case 377, Fig. 1A. Erect antero-posterior view of the abdomen shows a paucity of bowel gas. Opaque material is seen layering in the mid-abdomen, probably within the stomach and duodenal bulb. Fig. 1B. Lateral abdominal film shows the opaque material in the region of the stomach and duodenal bulb.

on the stool showed adult hemoglobin which was therefore attributed to swallowed maternal blood. He did well until the onset of vomiting at 8 days of age.

Abdominal films showed a paucity of bowel gas and the presence of calcific-like densities in the anterior mid-abdomen (Figs. 1A, 1B). No medications had been administered to the infant, and the origin of these densities was initially unknown. However, careful inquiry into the circumstances of feeding yielded the

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* Abbott

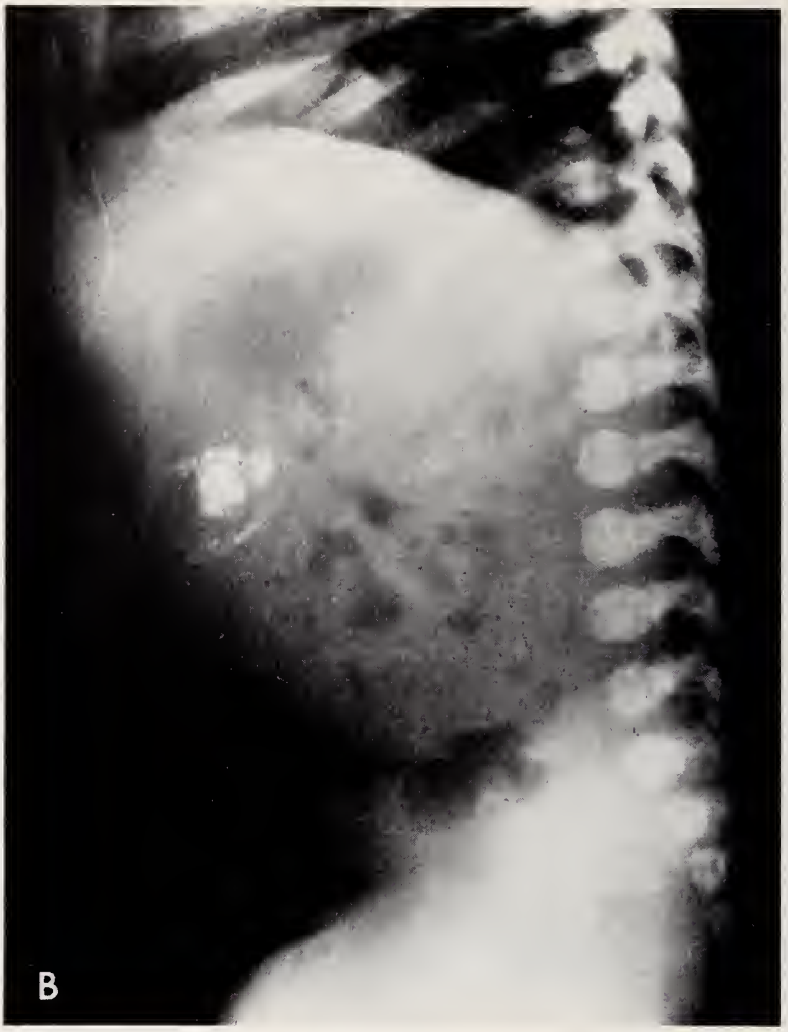


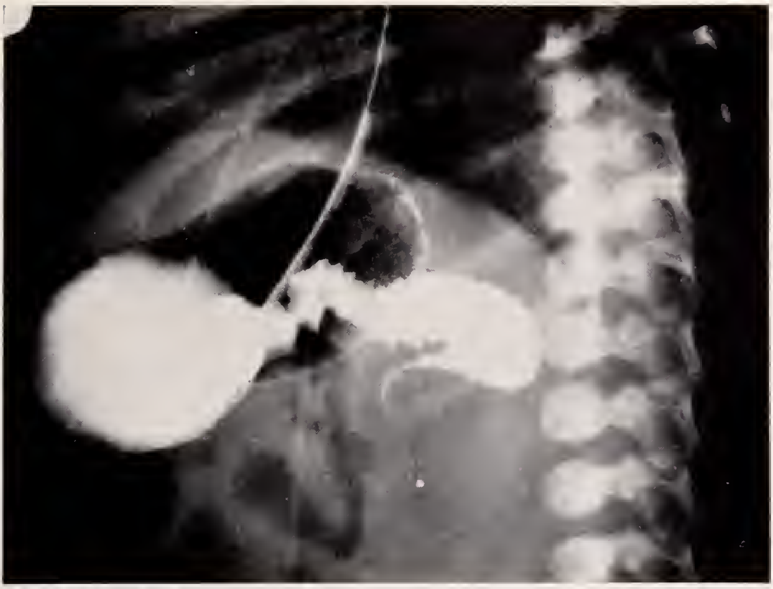
Fig. 1B

information that the baby was breast-fed, and the mother applied a bismuth-containing ointment (Mammol*) to her nipples prior to each feeding.

Upper gastrointestinal series showed obstruction of the second portion of the duodenum (Fig. 2). At surgery, a malrotation with Ladd's bands and midgut volvulus were found. The volvulus was derotated and the bands divided. The infant recovered uneventfully.

Discussion

The location of the opaque material in the stomach and duodenal bulb of this infant left little doubt that it represented ingested material even before its exact



Case 377, Fig. 2. Lateral film of the upper gastrointestinal series shows obstruction of the third portion of the duodenum which was due to Ladd's bands.

origin was known. This substance, if present in a more distal portion of the gastrointestinal tract, could well simulate an intraabdominal calcification.

Mammol is an ointment widely used by nursing mothers for the prevention of cracked nipples. The manufacturer's recommendations for use include the removal of excess ointment before each nursing. The ingredients are bismuth subnitrate (40%), castor oil (30%), anhydrous lanolin (22%), ceresin wax (7%), and balsam peru (1%). The radio-opaque bismuth may be detected in the infant's gastrointestinal tract if incompletely removed prior to nursing.

Acknowledgment

The editors wish to thank Dr. Donald Gribetz for permission to publish this case.

Case Report: INGESTION OF BISMUTH-CONTAINING OINTMENT BY A BREAST-FED INFANT.

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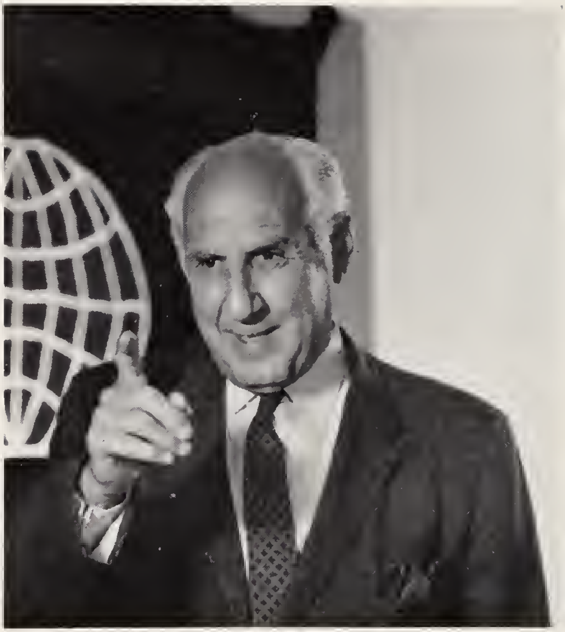

ALAN F. GUTTMACHER, M.D.
1898–1974

In Memoriam

ALAN F. GUTTMACHER, M.D.
1898–1974

Alan Frank Guttmacher was one of identical twin sons born on 19 May 1898 in Baltimore, Maryland, to Adolf Guttmacher, a leading Reform rabbi, and his wife Laura (née Oppenheimer), a distinguished social worker. He died at The Mount Sinai Hospital, New York on 18 March 1974 after an extended illness with chronic myelogenous leukemia. The intervening 75 years were filled with struggle, dedication and service, and as granted only to a few, accomplishment, recognition, and public esteem.

The public career of Alan Guttmacher is well known. A graduate of The Johns Hopkins University in 1919 and The Johns Hopkins University School of Medicine in 1923, he received his training in obstetrics at The Johns Hopkins Hospital, Baltimore, under J. Whitridge Williams, one of the deans of American obstetrics; in gynecology, at The Mount Sinai Hospital, New York, under Robert T. Frank, one of this country's pioneers in gynecologic endocrinology; and in forthrightness and dislike of hypocrisy from a long friendship with Henry L. Mencken, the Baltimore iconoclast. He was in the clinical practice of obstetrics and gynecology in Baltimore for almost a quarter of a century. He served as Chief of Obstetrics at the Sinai Hospital of Baltimore from 1943 to 1952 and rose to the position of Associate Professor of Obstetrics and Gynecology in the Johns Hopkins School of Medicine, establishing early a reputation for clinical excellence, personal compassion, progressive social concern, and a superb talent as an educator.

In 1952, Alan Guttmacher came to The Mount Sinai Hospital as the first Director of a combined Department of Obstetrics and Gynecology, being named at the same time Clinical Professor of Obstetrics and Gynecology in the College of Physicians and Surgeons of Columbia University. He introduced to The Mount Sinai Hospital and established what may have been for the first time in this country an obstetric service whose interest in and emphasis on maternal and fetal medicine presaged changes to come in the specialty in the next two decades. He established hospital programs on abortion, sterilization, and contraception which forced the medical establishment to confront and deal with these areas of patient care and served as models for similar programs throughout the country. His concern for the well-being of the individual, and his dream that each child born not only be in an optimal physical and mental state but should be wanted and loved by its parents, led Alan not only to initiation and support of medical research in these areas, but also to increasing involvement with voluntary family planning and social change, and in 1960 he left The Mount Sinai Hospital and the new Mount Sinai School of Medicine which he helped to found, to become President of the Planned Parenthood Federation of America, a position he occupied with honor, distinction and marked success until his death. He was in the forefront of the unended struggle which has seen the recognition of population growth as a global ecologic problem, the acceptance of responsibility by government for the provision of basic medical and family planning services, and the statutory acceptance of the right of a woman to determine the use of her own body. He was the author of many lay and professional books and journal articles and the recipient of numerous awards: The Lasker Award, 1947; the Bronfman Award, 1970; the Margaret Sanger Award, 1972; Doctor of Science (Hon.), Brandeis University, 1970; Doctor of Science (Hon.), Dartmouth College, 1970; and Doctor of Laws (Hon.), Lake Forest College, 1973.

In 1925, Alan Guttmacher married Leonore Gidding, a union which for 49 years was strong, fruitful, and supportive and stimulating to both partners. He was the father of three daughters (Mrs. Ann G. Loeb, Mrs. Sally Holtzman, and Mrs. Susan Green) and had two grandchildren. From this family constellation and from his innate nature grew a love, understanding, and respect for young people and a commitment to their education. Alan Guttmacher had a direct and personal influence on the medical education and career development of hundreds of young physicians, for each one of whom he took personal interest and responsibility. There are scattered today throughout the country a multitude of physicians practicing by what Alan taught, living and interacting with patients in accord with his example, and in their turn transmitting philosophies of medical excellence, scientific curiosity, social awareness, and personal compassion to succeeding generations of physicians. Alan F. Guttmacher was influential, as few men have been, in changing the direction and interests of his chosen profession, for which his colleagues, his students, and women throughout the world are grateful.

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Autoimmune Phenomena in Evolution of Systemic Lupus Erythematosus—Some Reflections on Its Interpretation as a Slow Virus Infection*

SYLVAN E. MOOLTEN, M.D.

The Continuing Dilemma: What Is Collagen Disease?

The term "collagen disease" has become so firmly embedded in the thinking of physicians that often its true meaning is lost to sight. To illustrate this point let me describe a hypothetical disease as a test of "audience reaction."

This hypothetical disease is:

1. A chronic disease of long duration marked by frequent remissions and exacerbations.
2. It is a systemic disease which produces inflammatory changes in many tissues, concentrated largely in the supporting connective tissues and blood vessels.
3. The disease is further characterized by the presence of: a) skin rashes; b) arthritis, which may at times simulate rheumatic fever; c) anemia; d) fever, which is often quite pronounced; e) generalized enlargement of lymph nodes; and f) occasional involvement of the kidney in the form of the nephrotic syndrome.
4. Diagnosis is confirmed by testing a sample of the patient's blood.

The almost universal response of the medical audience is to call out "systemic lupus erythematosus." Actually the identical description could be applied to a disease known better to physicians of an earlier era, namely, syphilis. In its evolution syphilis shares many features in common with systemic lupus, scleroderma, rheumatic fever, dermatomyositis, and sarcoidosis. We know the cause of syphilis, a spirochete, discovered more than half a century ago. For that matter we know the cause (or presumed cause) of rheumatic fever, the hemolytic streptococcus. We know also that dermatomyositis in adults is commonly associated with cancer. Sarcoidosis fulfills all the criteria of a collagen disorder even though the unit lesion is a miliary granuloma rather than diffuse inflammation.

In short, the category "collagen disease" is no more than a topographic map of disease, a description of the *location* of disease, and in no sense a diagnosis. The term "collagen disease" has begun to go into disuse because of the mislead-

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From the Department of Laboratories, Middlesex General Hospital, New Brunswick, N.J.

* Lecture delivered at Postgraduate Course, Middlesex General Hospital, New Brunswick, N.J., February 5, 1969.

Requests for reprints should be sent to: Sylvan E. Moolten, M.D., Roosevelt Hospital, Box 151, Metuchen, N.J. 08840.

ing implications that it represents allergy or autoimmunity to collagen. Instead the term "connective tissue disease" is being quietly substituted.

If our interpretation of syphilis were to depend upon its clinical manifestations, its pathologic features, and the serologic test, which is a test for a tissue antigen (beef heart antigen), one might even now speak of syphilis as a connective tissue disorder induced by autoimmunity. Only the saving grace of the discovery of the spirochete preserves us from such error.

Hypersensitivity Phenomena in the Evolution of Infection

At one time systemic lupus was considered a form of hypersensitivity disease, in fact an allergic disease. This arose from its frequent association with drug sensitivity and extreme sensitivity to exposure to the sun. No proof has ever been established of any type of allergy in the background of systemic lupus except as a provocative stimulus. Patients with SLE are usually free of asthma and other outspoken allergic disorders and eosinophilia is not part of the typical picture.

If we did not know that herpes simplex is caused by a specific virus we might be persuaded that herpes simplex is caused by some type of allergy. Herpes simplex infection is characteristically latent until the patient is exposed to intense sunlight or high temperatures. The list of provocative factors of herpes simplex is remarkably similar to that of systemic lupus, as indicated in Table I.

Autoimmune Phenomena and Systemic Lupus

In no other disease but systemic lupus erythematosus has the concept of autoimmunity been advocated with such assurance. Its frequent association with Coombs' positive acute hemolytic anemia, the LE-cell phenomenon, and the presence of antibodies against nuclear proteins all suggest the existence of some type of autoimmune mechanism in its pathogenesis.

If autoantibodies were the basic cause of systemic lupus, how then would one reconcile the occasional development of Coombs' positive hemolytic anemia

TABLE I

A Comparison of Factors Which Activate Herpes Simplex and Systemic Lupus

Herpes Simplex	Systemic Lupus
Sunlight	Sunlight
Cold drafts	Cold
Trauma, local irritation	Trauma, burns
Artificial fever, acute febrile illness	Hot environment
Typhoid vaccine	Typhoid vaccine
Anaphylactic shock, food allergies	Pollen injections, penicillin allergy
Drug reactions (sulfapyridine)	Drug reactions (gold, quinine, hydralazine, procainamide)
Menstruation	Menstruation
High doses of steroids	Pregnancy, oophorectomy
Severe emotional disturbance	Severe emotional disturbance

with an obvious infection such as infectious mononucleosis? Or herpes simplex? The patient with infectious mononucleosis has prolonged fever, in many instances for months, and in a few instances for six years or longer. A rash may be present. Aberrant immunologic responses are often present. As in SLE, false positive tests for syphilis are a common finding. The diagnosis of infectious mononucleosis is made by the demonstration of antibodies against cellular constituents, specifically red blood cells, i.e., the red blood cells of sheep, horse, or other species (heterophile agglutinins). We now have evidence which seems to indicate that a virus is implicated in infectious mononucleosis, i.e., the EB virus, and presumably responsible for its autoimmune and heteroimmune features.

The Nature of Autoimmunity

What then is autoimmunity and what is its significance in the disease picture? At present two theories are held, the "forbidden clone" hypothesis and, for want of a better term, what we might call the "scavenger" hypothesis.

The Forbidden Clone Hypothesis of Autoimmunity

This concept, proposed originally by Burnet, implies that a line (or "clone") of lymphocytes is left over from fetal life in "uncommitted" form, i.e., lacking "instruction" to participate in defending the body against foreign substances, such as foreign cells, foreign proteins, or other forms of antigen. Accordingly such lymphocytes are unable to distinguish between "self" and "non-self," i.e., between host cells and foreign cells such as might be introduced as a homograft. Later in life these lymphocytes spring into action, possibly spontaneously or in response to some irritant, and behave as hostile immunocytes against the tissues of their own host. They then attack the cells of the body, much as in the case of a graft rejection response. According to this concept, systemic lupus erythematosus results when the connective tissues of the body are the target of these lymphocytes.

According to Dameshek, normal lymphocytes may undergo a type of "brainwashing" or mutation and then may behave in the same manner as a "forbidden clone" and attack normal cells. Such mutation may arise either on the basis of viral infection, radiation, or the effects of certain drugs or chemicals. In his brilliant synthesis of this idea Doctor Dameshek further suggested that the same mutation which transforms normal lymphocytes into hostile lymphocytes to produce autoimmune phenomena may also underlie their eventual transformation into tumor lymphocytes, i.e., lymphosarcoma or chronic lymphatic leukemia.

The Scavenger Hypothesis

According to the second hypothesis, the attacking lymphocytes are normal lymphocytes. The transformation involves not the lymphocytes but the cells of the body which, in some way, become "foreign." Such alteration may result from any type of cellular injury such as injury by drugs, chemicals, ischemia, or infection.

One well-known example of such an autoimmune response is the condition referred to as the "postmyocardial infarction syndrome." In this condition a patient convalescing from myocardial infarction develops an acute syndrome several weeks later consisting of fever, chest pain, pneumonitis, and pericarditis which are associated with the presence of autoantibodies against heart muscle. The inflammatory changes that occur in the heart, lung, and other tissues in these patients possibly represent a mild, transient variety of graft rejection response. The same type of phenomenon may account for chronic thyroiditis developing after mumps virus infection of the thyroid. We must also keep in mind the distinction between autoimmune reactions instigated by lymphocytes and the changes produced by autoantibodies. Circulating autoantibodies may in fact produce little or no cellular injury. There is reason to believe that autoantibodies actually have a protective function. In experimental allergic encephalomyelitis produced by immunizing an animal against nerve tissue, the presence of autoantibodies for nerve tissue seems to protect against attack by lymphocytes, possibly by competing for the same binding sites on the surface of the target cells. In the absence of autoantibodies the lymphocytes apparently are unhindered in their destructive action against nerve tissue.

The original concept that autoantibodies cause cellular injury was based on the observation that antibody could be eluted from red blood cells in cases of hemolytic anemia and would be active against other red cells.

Acquired hemolytic anemia associated with a positive Coomb's reaction is by far the best known type of autoimmune disease. How then are autoantibodies aroused against red blood cells? A number of factors may be responsible by injuring the red cell and thereby rendering it "foreign" or antigenic. For example, both Mesantoin®, used in treating epilepsy, and Aldomet®, used in treating hypertension, may produce hemolytic anemia associated with a positive Coombs' reaction. Many viral infections, including infectious mononucleosis, rubella, viral hepatitis, and cytomegalovirus infection may be associated with hemolytic anemia. Even a mycoplasma (mycoplasma pneumoniae), which is the cause of primary atypical pneumonia, can elicit autoantibodies against red blood cells manifested in high titers of cold agglutinins for red cells. In some cases the patient develops Coombs-positive hemolytic anemia. The diagnosis of primary atypical pneumonia is commonly made by the demonstration of high titers of cold agglutinins.

Basically the development of autoantibodies for red cells represents, therefore, one mechanism for ridding the body of injured red cells. When we speak of "idiopathic" hemolytic anemia we are merely indicating our inability to demonstrate the primary cause of the red cell injury which incites autoantibody formation.

Immune Complex Disease

The concept of immune complex disease has begun to engage the interest of many investigators in recent years and has aided our understanding of a number of disorders hitherto attributed to "hypersensitivity", particularly glomerulonephritis and arteritis. Studies by Dixon, Germuth and their associates have provided evidence that an immune complex (i.e., antigen plus antibody) circulating in the blood may become deposited upon the surface of certain cells following which complement is attracted to the cells. The fixation of complement activates lytic enzymes, encompassing destruction of the cell even though it was no more than an "innocent bystander." For example, if bovine serum albumin is injected repeatedly into rabbits, the development of the immune complex (bovine serum albumin plus antibody against bovine serum albumin) in the circulating blood gives rise to glomerulonephritis and arteritis. In many respects this phenomenon mimics the lesions of serum sickness.

A similar phenomenon has been recently described as a complication of spontaneous infection of mice with the virus of lymphocytic choriomeningitis (LCM). This virus is prevalent in many colonies of ordinary house mice and every member of the colony is infected. For a long time it has been accepted that these mice are free of any disease manifestations caused by the virus. This has been explained by the fact that the LCM virus is introduced into newborn mice before they are mature enough to generate antibodies. Accordingly the virus multiplies in these mice and freely circulates in their blood and tissues. The lack of "immunologic competence" to develop antibodies protects these mice against inflammatory responses caused by combination of virus with antibody. However if blood from one of these mice is injected into an adult mouse of a virus-free colony, the animal develops acute illness and if it recovers will then possess antibodies in high titer and the virus disappears completely from its blood.

More recently the interesting finding has come to light that antibodies do actually appear early after infection of newborn mice with LCM virus but in such low titer that no obvious reaction is recognizable in the form of disease. These small amounts of antibody in the presence of great excess of viral antigen are insufficient to clear the blood of virus but give rise to a constantly rising level of immune complex in the blood, which is slowly deposited in the glomeruli of the kidney. Eventually sufficient immune complex accumulates in the glomeruli to produce fatal glomerulonephritis.

When one considers the enormous mass of red blood cells and the vast surface they present to the plasma in which they circulate, it is easily understood why red cells should be more vulnerable than any other tissue to the localization of circulating immune complexes. Actually this may serve a protective function. Red cells, by picking up immune complexes, protect other tissues against these complexes at the price of their own destruction. On that basis autoimmune hemolytic anemia may represent the body's first line of defense against immune complex disease. Red cells coated with immune complex will, of course, give a positive Coombs' test although the antibody in such cases is not an antibody for red cells but for some other antigen, the red cell being merely the "innocent bystander."

Factors Governing the Localization of Immune Complexes

Localization of immune complexes within a tissue depends on many factors. If immune complexes develop slowly and gradually over a period of weeks, months, or years, more and more immune complex may escape the red cell protective barrier and become deposited in glomeruli and blood vessels, as in the case of the LCM-infected mice. Perhaps their localization in these sites may depend on hemodynamic stresses, as suggested by Germuth. For example, the endothelial lining of the glomeruli is subject to the stress of arterial pressure magnified by hemoconcentration as a result of glomerular filtration of water. Arteries may be affected because of the pulsatile stress of systolic arterial blood pressure.

Along the same lines we can easily understand why an immune complex excited by the hemolytic streptococcus may become selectively localized in the heart, especially in the left atrium and left ventricle where pressures are greatest, hence the predominant localization of rheumatic valvulitis in the mitral and aortic valves rather than in the pulmonic and tricuspid valves.

The effect of mechanical stress presumably accounts also for the localization of immune complexes in joint tissues, not only in rheumatic fever but also in rheumatoid arthritis. The periarticular nodules of rheumatic fever and of rheumatoid arthritis are characteristically located about knuckles, elbows, and other sites subjected to frequent mechanical trauma.

Localization of immune complexes may occur massively in the lung in rheumatic coal miners. Large quantities of dust retained in the pulmonary tissue appear to have a localizing influence in patients suffering from rheumatoid arthritis so that they develop enormous rheumatoid granulomata in the lung (Caplan's syndrome). In fact, a report appeared in the *New England Journal of Medicine* describing rheumatoid nodules on the face simulating skin cancer in a patient with rheumatoid arthritis, their size and location coinciding with the sites of pressure of eyeglasses.

This mechanism of localization of immune complexes has been known since 1920 when Dr. John Auer at the Rockefeller Institute experimented with rabbits sensitized with repeated injections of bovine serum albumin. Where he rubbed the ear of a rabbit with xylol, the sensitized rabbit developed intense inflammatory reaction with vesiculation going on to superficial necrosis instead of the mild erythema which appeared in the control rabbit. (The Auer phenomenon should receive more attention by physicians in their interpretation of the pathogenesis of a number of disease syndromes.) One can in fact regard the Auer phenomenon as perhaps an explanation for the localization of the butterfly eruption of the face in systemic lupus erythematosus, representing the localization of a circulating immune complex through sunlight.

Systemic Lupus Erythematosus Considered as an Infection

These several considerations are important in the case of systemic lupus erythematosus, many facets of which are strongly suggestive of an infectious disorder. For example, SLE has a protracted course with systemic manifestations which are reminiscent of such infections as syphilis and infectious mononucleosis, systemic infections involving connective tissues. Systemic lupus may pursue a sluggish, even latent, course for long periods of time as in the case of herpes simplex, a disease acquired usually in early childhood but remaining hidden until provoked into acute activity under the stress of sunlight, pregnancy, allergic reactions, or emotional stress.

Many of the serologic abnormalities which characterize systemic lupus are paralleled in other infections. For example the false positive test for syphilis is very common in SLE just as it is in infectious mononucleosis and following vaccination against smallpox. Indeed, in any patient with persistent biologic false positive tests for syphilis, we should think at once of SLE as the most likely basis. The serologic test for syphilis, as already indicated, represents a hetero-immune response against beef-heart antigen. In SLE antibodies may develop against red cells, accounting for occasional Coombs' positive hemolytic anemia, just as in the case of infectious mononucleosis, infectious hepatitis, and mycoplasma infection.

As in the case of these infections, SLE is also diagnosed by testing a sample of blood from the patient either for antibodies against red cells (positive Coombs' test) or the presence of a specific reaction against leukocytes (the LE-cell phenomenon), or against the products of cellular breakdown, particularly nucleoprotein (antinuclear factor). Finally, prolonged antigenic stimulation by the LE factor and by the products of cellular breakdown produces an excessive immune response in which the circulating antibodies eventually become altered by the antigen with which they combine so as to become, in effect, foreign proteins, exciting in turn an autoantibody composed of 7S gamma globulin. This phenomenon is applied in clinical diagnosis in the form of the latex agglutination test. It is positive in rheumatoid arthritis and in a large variety of other prolonged infections including syphilis and subacute bacterial endocarditis as well as in SLE.

The parallel with rheumatic fever, which has brought the latter disease into the category of "connective tissue disease" brings to mind the possibility that rheumatic fever may in fact represent an immune complex disease involving antibodies against streptococcal antigens with secondary deposition in the heart, joints, skin, lung, and blood vessels.

Another similarity between SLE and infectious mononucleosis is the frequent presence of atypical lymphocytes ("virocytes") in the blood which may be indistinguishable from the atypical cells of infectious mononucleosis.

Enlargement of the lymph nodes and spleen is a characteristic feature of SLE just as in the case of infectious mononucleosis. The lymph nodes in SLE may become necrotic so that in some instances the findings in the lymph nodes may suggest typhoid fever or tularemia. In extreme cases the nodes may even undergo a type of caseation. This may account for an old theory, long since discarded, that SLE was a form of tuberculosis.

Because much of the impact of SLE occurs in lymph nodes, bone marrow, cells of the connective tissue (perivascular histiocytes, fibroblasts) and blood vessels it is not surprising that the disease has won for itself the special designation of "connective tissue disease." we might regard the disease, therefore, as the manifestation of a "mesenchymotropic" infective agent. The lesions in the kidney, on the other hand, may represent a secondary phenomenon induced by immune complexes formed either against the causative agent itself or against products of cell destruction.

The Concept of Slow Virus Infection

We are accustomed to regard viral infections in general as acute self-limiting infections with relatively short incubation periods, usually under a month, and rapid proliferation of virus just before the acute phase of the disease. From then on, with the appearance of specific antibodies, the virus disappears promptly. This is the picture characteristic of measles, poliomyelitis, varicella, and a host of other viral infections.

The idea of a progressive pathologic process of long duration resulting from viral infection is strange, almost unique in virology. Even so, we have examples in such diseases as infectious mononucleosis. As already mentioned, infectious mononucleosis may run a prolonged course which in a few instances may persist for as much as six years. Infectious hepatitis may also progress from the acute into subacute and chronic forms. Cytomegalic inclusion disease of newborn infants may continue in active form for several months and produce progressive damage of the brain, liver, kidneys, lungs, and other organs. Several examples of prolonged progressive viral infection are found in other species. The example of lymphocytic choriomeningitis infection in mice has been mentioned. In this disease the newborn mice are infected but remain clinically free of disease until adult life when they may die of glomerulonephritis.

Equine Infectious Anemia

This viral infection of horses, also known as "swamp fever," is characterized by periodic fever, anemia with positive Coombs' reaction, and extensive injury of the bone marrow. The blood develops a high titer of gamma globulins. The lymph nodes undergo generalized enlargement and necrosis followed by regeneration and excessive proliferation of lymphocytes. In some instances the lymphocytic proliferation may advance to a stage which mimics lymphoma.

In equine infectious anemia evidences of immune complex disease are present in the form of fibrinoid necrosis of arteries and periarteritis. The renal glomeruli undergo progressive obliteration.

Aleutian Mink Disease

This viral infection of Aleutian minks has come into the spotlight recently because of its many similarities to "collagen disorder" of man. These animals are infected with a virus which produces leukopenia, thrombocytopenia, and increase in gamma globulins in the blood. Plasma cells are stimulated to proliferate to a degree which may mimic myeloma. Immune complex disorder is present in the form of glomerulonephritis and arteritis. Hepatitis and interstitial pneumonitis are common.

The Lupus Syndrome of NZB Mice

Perhaps the most interesting syndrome of all in this category is the lupus-like syndrome of a strain of mice found in New Zealand. The New Zealand black mouse (NZB strain) is affected from birth by a disease which mimics human SLE in a surprising manner. All the members of an affected colony are diseased, but females are affected more severely than males. Because the disease seemed to be limited to this particular strain of mice and to related hybrid strains, it was believed to be a genetic disorder. The newborn mice appear healthy but as they approach adult life they develop hemolytic anemia with a positive Coombs' test (autoimmune hemolytic anemia). Even more interesting is the frequent development of glomerulonephritis which in all essential respects parallels the nephritis found in human SLE. Furthermore the blood of these animals can be shown to produce a positive LE-cell phenomenon. It is hardly surprising, therefore, that the example of this disease in mice has been commonly cited as proof that SLE in man is also an autoimmune disease based on genetic abnormality or predisposition. One of the interesting byproducts of the NZB disease is the intense proliferative activity of lymphocytes. Eventually one-third to one-half of the mice develop lymphosarcoma or chronic lymphatic leukemia, embodying Dameshek's concept of immunoproliferative disease evolving from autoimmune disorder to lymphoid neoplasm. The fact that all the phenomena of disease in NZB mice are the result of viral infection became known only in the last few years. In a series of papers published by Mellors and his associate in the *Journal of Experimental Medicine* observations were reported in which cell-free filtrates from affected NZB mice could be shown to reproduce the disease in every respect when injected into the newborn of an entirely different strain of mice. The viral nature of the infective agent was also confirmed by electron microscopy.

The special characteristics of this group of viral infections, e.g., LCM infection in mice, equine infectious anemia in horses, Aleutian mink disease, and the LE-like disease of NZB mice, have led to their inclusion in a new category of virus infection, "slow virus infection." As defined originally by Sigurdsson, who reported observations of similar type in sheep, slow virus infection is characterized by the following:

1. A very long latent period.
2. Prolonged course with progressively increasing disability ending often fatally.
3. Involvement either of a single organ or of a single tissue system.

Thus far only one disease has been convincingly established as a slow virus infection of man, Kuru. This disease is a chronic encephalitis occurring among New Guinea natives who live in the highlands. These primitive people develop a peculiar type of encephalitis in adult life producing madness ending in death. A variety of theories were first proposed to account for the disorder, genetic abnormality, hysteria, and, of course, autoimmune disease. The fact that cannibalism existed in this tribe led to the thought that the eating of human brains was the inciting factor behind autoimmunity. Recently it has been shown that the disease is actually a virus infection and is transmissible to chimpanzees. Because of its long latent period and progressive course, Kuru is quite clearly eligible for inclusion in the slow virus category.

Several other diseases have been proposed as candidates for classification as slow virus infections. Among the likely candidates is multiple sclerosis, which, according to some observers, may represent a continuing slow virus infection originating in childhood measles. Even cancer has become subject to reconsideration as a slow viral infection. There are many indirect hints of this possibility even in human cancer. For example, a number of syndromes are associated with malignancy which in many respects suggest the presence of a virus or virus-like agent inciting tissue damage or immune complex disease. These are grouped into a loose category, "paraneoplastic disorders."

One of the most common paraneoplastic disorders is multifocal leukoencephalopathy. This is a type of encephalitis, affecting chiefly the cerebellum. which is found in many patients with cancer, particularly Hodgkin's disease. Recently particles resembling virus particles have been demonstrated by electron microscopy in the brain of such patients. Another paraneoplastic disorder which in many respects suggests immune complex disease is dermatomyositis. When this occurs in adults it is most commonly associated with hidden cancer and may appear long before there is any sign of cancer. Other cancer-associated paraneoplastic disorders include acanthosis nigricans, erythema gyratum repens, and palmar keratoses. Ichthyosis has been found to develop in a fair proportion of patients with Hodgkin's disease.

One of the most interesting aspects of cancer in general and of chronic lymphatic leukemia and Hodgkin's disease in particular is their association with anemia. In general the anemia of cancer has been shown to be the result not of an autoantibody or of an abnormality of the red blood cell but of some factor in the circulating blood which becomes adsorbed to red blood cells. Evidence has been found which implicates a filterable agent transmissible to animals and in all likelihood a virus. In the case of chronic lymphatic leukemia and Hodgkin's disease the factor responsible for anemia may give rise to the picture of acute hemolytic anemia with a positive Coombs' test.

Systemic Lupus as Slow Virus Infection

Can one interpret SLE as a slow virus infection based on the examples given? This is highly probable for many reasons. The example of the NZB mice is strongly suggestive. Originally the NZB mouse disorder was considered a genetically determined disease of mice. In connection with the presumed genetic background of human SLE there are abundant evidences of its familial nature. For example, in the families of many patients with SLE there are other members who have either chronic discoid LE, rheumatoid arthritis, hypergammaglobulinemia, or antinuclear factors in the blood. More than 40 families are on record in which more than one member was afflicted with either SLE or chronic discoid LE. In 23 of these families siblings were affected, sisters in 19 families, brothers in one family, and brothers and sisters in three families. In 15 of these families the disease affected identical twins. Parents and children were also affected in 21 other families. In one of these a father and a child were affected. In the remaining 20 the mother and child were affected.

This striking difference between father-child and mother-child incidence may be quite important. Obviously sex alone is not the only factor, as shown by the fact that the affected children included boys as well as girls. What may be more likely the case is that the infection was present in the mother before pregnancy, possibly in latent form and activated by pregnancy so that virus was transmitted transplacentally to the fetus. This type of infection ("vertical transmission"), as shown by Ludwik Gross, is not uncommon in certain viral diseases of other species, e.g., bovine leukemia and the milk-borne mammary tumor virus of Bittner. In the case of SLE in man vertical transmission may account for the mother-child and sibling incidence of the disease and the fact that no evidence has ever been adduced that the disease is contagious between adults, e.g., husband and wife. Perhaps in SLE we are dealing with a familial virus disorder transmitted at or before birth much as in the case of lymphocytic choriomeningitis of wild mice.

Other indications of slow virus infection in SLE include the long latent period of the disease. Many of these patients have a childhood history of delicate health and excessive sensitivity to fatigue, chilling, drugs, and emotional stress. Many of these children suffer from muscle aches and pains, anemia, and occasional attacks of fever of unknown origin. These symptoms may represent minor premonitory outbursts of the ongathering infection. Overt SLE usually comes to light in adolescence or early adult life either explosively or insidiously. In some instances it may not emerge until old age.

The more dangerous manifestations of SLE often follow some type of provocative incident. For example a patient complaining of rheumatoid pains and low grade illness may be advised to make a trip to Florida and lie in the sun. Or he may be given gold injections or treated for focal infection. Any of these may provoke the acute outburst of the disease in dangerous form. In some instances certain drugs may be responsible. As already mentioned, hydralazine, gold, quinine, and procainamide (Pronestyl®) are common provocative factors. When these are withdrawn the patient may revert into the quiescent form of the disease or the disease may continue to progress. A severe emotional upheaval, for example, a disappointment in love, may be the provocative factor. Pregnancy seems to play an important part in the activation of the acute disease, sometimes in fatal form, often preceded by spontaneous abortion.

These acute flareups may be of extreme severity. They may take a fulminant form in which the patient becomes acutely ill with a typhoid-like picture marked by high fever, profound leukopenia, extreme prostration, and the patient may die within as short a time as two weeks from onset. In other cases the acute disease pursues a somewhat slower course, which is more like the textbook picture of SLE, i.e., the typical eruption on the cheeks and elsewhere, fever, and arthritis. Hyperacute and acute types which end fatally in the first attack may reveal very little at autopsy which is characteristic of SLE. On the other hand in the more prolonged and recurrent types, i.e. subacute disseminated SLE, in which immune complex phenomena are probably operative, the patient will eventually die of lupus glomerulonephritis and autopsy will reveal such associated lesions as vasculitis, pneumonitis, atypical verrucous endocarditis, pericarditis, and pleuritis.

More benign types of SLE may elude exact diagnosis for a long time. For example the disease may first emerge in the form of an attack of acute hemolytic anemia with a positive Coombs' test, relieved or cured by cortisone treatment. In other cases thrombocytopenic purpura may appear and may be cured by splenectomy. Examination of the spleen histologically may provide the first clue that SLE was responsible if it reveals the characteristic "onion peel" perivascular fibrosis of the arterioles. In some instances following splenectomy for purpura the patient may later develop acute hemolytic anemia. Obviously, the latter cannot be attributed to hypersplenism. In other cases nephrosis may be the first manifestation of SLE. In fact SLE seems to be the most common cause of nephrosis in young women.

Another manifestation of hidden SLE is a form of hepatitis which occurs especially in young women, often associated with marked plasma cell infiltration of the spleen, lymph nodes, and the connective tissue of the liver. In some cases there may be few or no other aspects of SLE. In other cases arthritis, glomerulonephritis, and a positive LE test may be found, confirming the lupus origin of the disease.

In some instances the presenting picture of SLE may be chronic encephalitis with psychotic manifestations or epilepsy. Other expressions of hidden SLE include ulcerative colitis, Sjögren's syndrome and Raynaud's syndrome. In some instances the infection announces itself in recognizable form from the first as chronic discoid LE. In such cases the patient may remain in good health otherwise or he may develop minor systemic manifestations. In certain cases chronic discoid LE may evolve into subacute or acute systemic LE of the classical type and may also revert back eventually to the chronic discoid form. The disease may remain clinically latent in certain cases until late adult life and then appear under the guise of rheumatoid arthritis. Only if a positive LE-cell test is obtained can one then have reason to suspect SLE as its basis.

One should think of SLE, therefore, as an "iceberg disease" in which only a small portion of the basic disorder may emerge at a given time rather than the complete classic picture of high fever, butterfly rash, arthritis, and anemia. Largely because of the discovery of the LE-cell phenomenon we are now able to recognize the many faces of SLE. One of the characteristics of SLE is its tendency to undergo remission. In some cases the course of SLE is marked by repeated exacerbations and remissions. Spontaneous remission has been reported to occur from a crisis of fulminant SLE in which the patient seemed almost moribund.

The effect of cortisone therapy deserves comment. In the acute and fulminant types cortisone obviously may be life saving. In subacute forms of the disease, in which immune complex nephritis develops, cortisone is not always of benefit and may actually increase the severity of the patient's edema and hypertension. Treatment with cortisone has been reported by Dubois to diminish the patient's chances of spontaneous remission. He advised a fairly long period of expectant treatment in milder cases before resorting to steroids. One should recall the vogue which once existed for treating viral hepatitis with steroids. In fulminant hepatitis, to be sure, an occasional patient may be salvaged by treatment with massive doses of steroids. In ordinary cases of infectious hepatitis, steroids have been shown to increase the tendency of the disease to persist in subacute and chronic form whereas without steroids it is more likely to undergo complete resolution.

SLE as a Paraneoplastic Disorder

An interesting parallel to the late development of lymphoma in the SLE syndrome of NZB mice has been observed also in a number of instances of SLE in man. Several observers have reported instances in which SLE has been associated with the late development of Hodgkin's disease, chronic lymphatic leukemia, lymphosarcoma, or Waldenström's disease. This may indeed represent an example of human malignancy which in all likelihood can be attributed to an underlying viral infection.

The Role of Sex Endocrine Factors in SLE

As in the case of the NZB mice, females are predominantly affected in human SLE. The reason for relatively selective involvement of the female is not entirely clear. It is known that tuberculosis runs a less favorable course in females than in males, both in humans and in rabbits. Hormonal factors are also suspect in this disease. Menstrual fever is common in tuberculosis of young women and pregnancy is a well-known cause of flareup of tuberculosis. Females have been shown to be more susceptible to such viral infections as influenza and poliomyelitis.

There is reason to believe that progesterone may be the underlying factor in the predisposition of female subjects to SLE. Progesterone has been shown to behave as a "spreading factor" which facilitates the spread of infection in the tissues, possibly by increasing the permeability of tissues to minute particles such as viral particles.

In SLE the predominance of females is sharply limited to a certain period of life, the period between the menarche and the menopause. In that age group the ratio of females to males is in the order of 3:1 to 6:1. However in children before puberty there is no sex difference, male children being affected in the same degree as female children. The same is true of older adults, the ratio between male and female patients with SLE being about even.

Specific Evidence for SLE as a Viral Infection

In 1948 an Italian investigator, Bergamasco, reported interesting observations suggesting that the blood of patients with SLE contained a virus-like agent which could be passaged in embryonated eggs. Many of his observations were strikingly similar to our own, which began in 1950 when Ellen Clark and I began our studies on blood-borne viruses and their relation to hemolytic syndromes. This work, which was performed at St. Peter's Hospital in New Brunswick, was undertaken on the premise that viruses with an affinity for red blood cells could in some instances be detected in the circulating blood by inoculating chick embryos with samples of venous blood. This proved to be the case in a number of conditions, including SLE, infectious mononucleosis, Hodgkin's disease, and certain other disorders. In the case of SLE, the agent produced typical virus-type plaques on the chorioallantoic membrane of the infected

TABLE II

Red Cell Agglutination with Immune Sera against Virus-like Agents Isolated in Cases of Systemic Lupus Erythematosus

	Positive Tests	Total Tests	Percentage Positive
Hodgkin's disease			
1950–1953	0	95	
1965–1968	0	178	
		273	0
Leukemia LSa			
1950–1953	0	33	
1965–1968	0	145	
		178	0
Carcinoma			
1950–1953	0	20	
1965–1968	0	51	
		71	0
Systemic lupus erythematosus			
1950–1953	34	60	
1965–1968	11	41	
	45	101	44.4
Infectious mononucleosis			
1950–1953	0	58	
1965–1968	0	28	
		86	
Normal controls; other diseases			
1950–1953	0	271	
1965–1968	0	124	
		395	0

chick embryos and could be passaged serially many times from egg to egg. Electron microscopy was performed in one instance and demonstrated virus particles which measured approximately the size of a myxovirus such as influenza. In some of the early studies it was possible even to induce the LE cell phenomenon by incubating normal leukocytes from a healthy person with the egg fluid from an infected egg. In one experiment a rabbit which had been inoculated with the virus and maintained on cortisone to prevent it from developing immunity gave a positive LE-cell phenomenon. Thus far the nature of this virus has not been identified.

The most instructive and persuasive proof of the viral nature of SLE was based on studies conducted initially at St. Peter's Hospital with the help of Miss Clark and later at this hospital with the help of Mr. Miklos Milhalyfi. The method we used in both studies was a serologic technique of sharp specificity employing immune serum produced by repeated inoculation of the SLE egg-passage virus into healthy rabbits. The immune rabbit serum was first shaken with normal red blood cells of all blood types available in the blood bank so that it would have no agglutinating activity on normal red blood cells. The rabbit serum was then tested in serial dilution against the red blood cells of patients with a large variety of diseases. It was found that in more than 40 percent of patients with systemic lupus their red cells were agglutinated by the rabbit serum. Not a single case was observed in which the red cells from patients with other diseases or from healthy people gave a positive agglutination with the LE rabbit serum, as indicated in Table II. Thus out of 101 tests of blood from patients with proved SLE, 45 gave a positive agglutination test with the specific anti-LE immune serum, yet not a single one of 1003 control samples gave a positive agglutination.

Summary

In summary, therefore, there is reason to believe that systemic lupus erythematosus is a slow virus infection, probably arising in certain families on a congenital basis, i.e., transmitted in utero. According to many evidences the virus remains hidden in incomplete form within the cells of the body until induced to activity as complete virus by some type of flaring factor. The latter may be any one of a variety of nonspecific provocative factors, especially sunlight, but also various types of stress or allergy, resulting in emergence of the virus in a form capable of causing cellular injury with consequent inflammation and a variety of secondary immune responses, including immune complex disease, autoimmune phenomena, elevation in gamma globulins, rapid sedimentation rate, biologic false-positive tests for syphilis, Coombs' positive hemolytic anemia, and certain other perverted immune responses. The virus is probably mesenchymotropic in nature with specific affinity for lymphoid tissue, bone marrow, and other cells of mesenchymal origin.

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Family Psychiatric Treatment

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As Freud developed his psychological theories, he treated a single patient, alone in a face-to-face setting, with his hand on the patient's brow. Relatively rapidly, Freud's brilliant psychological observations and insights evolved hypotheses and altered technique. The position of the therapist behind the recumbent patient became standardized. The fundamental rule of free association, the transference relationship, and the analysis of dreams were established as cornerstones in reaching the patient's unconscious processes. With further knowledge as to the complexity of treatment and with the occurrence of different types of emotional problems and dissatisfaction with some of the results of therapy, there have been attempts at modification of the technique. Psychoanalytic thought has been in constant flux as clinical experiences have given birth to new theories, which have been tested, ultimately creating additional or modifying concepts. The psychoanalytic setting itself has changed. The one-to-one therapeutic relationship has been supplemented by group therapy and, in the treatment of the disturbed child, mother-child therapy. The purpose of this paper is to describe another modification, that is, the treatment of the family as a unit.

The principles of the psychotherapeutic methods utilized in family psychiatric therapy stem preponderantly from the psychoanalytic principles of Freud. Of Freud's theoretic contributions, the following are considered significant (1):

1) The concept of instincts, with their internally and somatically (genetically) derived sources. However, as stated by Erikson (2) in *Childhood and Society,* "the drives man is born with are not instincts, nor are his mother's complementary drives instinctive in nature. Neither carry in themselves the patterns of completion, of self-preservation, of interaction with any segment of nature. Tradition and conscience must organize them. Man's inborn instincts are drive fragments to be assimilated, given meaning and organized during a prolonged childhood by methods of child training and schooling, which vary from culture to culture and are organized by tradition."

2) The elucidation of how hereditary factors and experience determine not only what needs must be satisfied but also what adaptations can develop to assure the satisfaction of the needs. As emphasized by Ackerman (3) "the channels of expression of physiological needs are organized by the social interaction of child and parent which, in turn, reflects the personality of the parent, and a typical interpersonal relationship of the family group." The child-mother interaction can only be evaluated in the frame of the psychosocial structure of the larger unit, the family. Cultural influences are transmitted to the child through the parents acting as cultural carriers. The family is a psychological unit of society, the cradle of personality. Only in the time–space continuum of

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the social (family) development of personality can unconscious mental phenomena achieve meaning.

3) The concept of a danger signal, anxiety, warning of the threat of excess excitation arising from inside or outside the organism and threatening its safety.

4) The concept of defense (more properly, adaptation), that is, the capacity of the psychic apparatus to initiate both psychic and physiological processes intended simultaneously to avert dangers and assure satisfaction of instinctual needs. However, aggression is no longer conceived of as a death instinct, but rather as a defense, a means of control of environment, counteracting frustration and anxiety, and asserting the identity of the self in interpersonal situations.

5) The elucidation of the topography (id, ego, and superego) and economics of the psychic apparatus and the proof of the existence of unconscious mental forces. In this context, in family therapy, perhaps another structure of the psychic apparatus may be conceived, that is, *the family*, what it is, what it means to each member, how it influences the economy of the unconscious dynamics of each member, each interreacting with the other.

6) Freud stressed the orifices of the body, the skin, the activity of internal organs and muscle systems, as zones of experiential pleasure and avoidance of pain. However, they may be somatic agencies for the interchange of energy between the inner and outer environment, as well as subverbal means of interpersonal communication. The language of the body conveys messages to other persons as to a dominant affective mood, states of need, pleasure, or apprehensions of danger.

7) Individual therapy is centered about the transference phenomenon, the core of which is the child-parent pattern. But, in individual therapy, the transference processes are not easily matched against the reality of contemporary family processes. For an accurate understanding of the unconscious and of transference, these dynamics must be matched, not only to the realities of the analyst-person, but also to the conscious organization of the patient experience, the total integrative pattern of personality, and the prevailing interpersonal realities of contemporary family life. This is vividly seen in the family interview. Not only does the family therapist use the transference phenomenon as it presents (father-therapist; mother-therapist; child-therapist), but he can directly demonstrate the patterns in its original setting among the family members, child-parent, and even parent with his own parent, if the latter is a member of the family group.

The expression 'family group' consists of all who live together as a natural living unit under one roof. Usually this means father, mother and children, but may include other persons who have a functional participational role in the day-to-day life of the family group. It may, therefore, involve a grandparent, an aunt, a maid, or even a homemaker. In essence, then, the unit of interview comprises all those persons who share the identity of family and whose behavior is influenced by circular interchange of emotion within the group.

Family treatment is a natural level of entry into human relations. A patient does not get sick alone, nor does he get well alone. An individual learns about handling love, hate, anger and fear in his nuclear family. Sullivan (4), working with schizophrenics, saw that the patient viewed the hospital staff as an extension of his own family and he emphasized the importance of the hospital family, that is, the physician, nurse, and aides.

The individual member of the family is not to be lost as an individual. Gomberg (5) aptly stated, "We must not choose between a concept of the family and the psychology of the individual: it is through a balanced understanding of the interrelation between the two that we can achieve the most meaningful understanding and the most effective treatment." Treatment must be patient-oriented. The most common objection heard against family-type therapy is that it is too complicated, that there are too many transference reactions, the therapist is incapable of grasping all that is going on concomitantly, that an individual person's privacy is threatened and it is too great a factor in resistance, that it is too threatening to the fragile minds of children. Experience has demonstrated that the above objections are usually rationalizations of individuals who are either ignorant of the technique or frightened by the openness of the method. Nevertheless, it is felt that, with knowledge and training, therapists can overcome all these factors. There are indications and contraindications for individual psychoanalysis, reparative therapy, group therapy, and family therapy. Under optimum circumstances, experiences have already demonstrated that family therapy is an effective technique.

The central concern for the clinician must be the connection between the interpersonal conflict in family relationships and the intrapersonal conflict in the mind of one member. The family conflict, when intralocked in the mind of one person, cannot be solved. Intrapsychic conflict must be activated and reprojected into the field of family interaction. Family treatment conceptualizes expressions of conflict, suffering, and disablement, not in isolation, but rather within the network of the person's most significant and intimate relationships. Instincts, drives, needs, temperament—the various expressions of the inherited factors—are considered in the context of an ego psychology derived from the struggle for survival of a biologically helpless infant as it grows up in its natural habitat—the family. The emphasis in this orientation is on an ego psychology interacting with a person's inherited drives—becoming part of the unconscious mind and ultimately determining behavior. This frame of reference implies that an individual emotional disturbance cannot be evaluated or treated efficiently in solitude. Whatever is deviant in an individual's behavior needs to be visualized as a symptom of the psychopathology of the family group.

The essence of family treatment is the therapist's understanding and utilization of the lifelong unconscious factors which have mainly developed from family interaction and making them emotionally meaningful in current problems.

The role of the therapist, in general, is an active, open, forthright one. There is no question of anonymity; he acts as a catalyst, dissolving the barriers of communication. He tries to bring about a useful, appropriate emotional interchange, in which the family members are encouraged to take part in the struggle to reach and understand important areas of unconscious conflict. Through the transference phenomena and the direct activation of the parent-child behavior patterns, the therapist demonstrates the ineffectiveness and harmfulness of the family's unconscious habitual ways and control and defense: their alienation and fragmentation in family relationships, the multiple levels of mistrust, despair, fear and hatred. Gradually, step by step, he activates an awareness of new avenues of sharing, new kinds of intimacy, new levels of identification, and he mobilizes a realignment of emotional relationships in the family in the direction of mental health.

Granted a theoretical and psychological frame of reference and a technique, the essential ingredient in psychiatric therapy is the quality (or perhaps the fit) of the doctor-patient relationship. More precisely with respect to the therapist's personality, the critical ingredients are warmth, empathy, openness, acceptance, courage, and always the ability to listen with a third ear. Ackerman was one of the early investigators who used video tape and one-way mirror supervision. Thus films of family sessions are available for those interested in learning by literally watching a family psychiatrist.*

Conclusion

When indicated, the family approach to psychiatric problems is feasible and effective.

Family technique, as taught by Ackerman, utilizes the concept of the unconscious and the topography and economics of the psychic apparatus, anxiety as a danger signal, the process of adaptation (or myriad defenses) of the developing individual, and the "transference phenomenon."

A more active role of the therapist is stressed, especially the therapist's willingness to accept the multifaceted, multileveled interactions which take place in the family therapeutic setting.

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Gamna-Gandy Body in a Cardiac Myxoma

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Abstract

This report of Gamna-Gandy bodies in a left atrial myxoma suggests that fibrosiderotic nodules previously reported in this region of the heart may represent an unusual degenerative change in a cardiac myxoma.

On several occasions, Gamna-Gandy nodules have been reported within organs other than the spleen (1, 2). The heart has been recently mentioned as the site of such a fibrosiderotic nodule (3). The pathogenesis was attributed to small focal hemorrhages within the atrial wall, with subsequent degeneration and deposition of calcium and iron salts on the connective tissue fibers. We recently encountered a patient who had a mass attached to the wall of the left atrium that contained similar Gamna-Gandy structures. The morphologic findings suggest that fibrosiderotic nodules in this location represent an uncommon degenerative change within a cardiac myxoma.

Case Report

A 57-year-old white man with a three-month history of angina pectoris was admitted for cine-cardioangiography. Extreme stenosis of the right main coronary artery was noted. Thrombectomy and angioplasty were performed under total cardiopulmonary bypass, hypothermia and perfusion of the right coronary artery. After five uneventful years he was readmitted with severe chest pain, which was diagnosed as a myocardial infarction. He developed complete atrio-ventricular block and expired on the fourth hospital day.

At autopsy, the heart weighed 500 gm and the left ventricular myocardium was hypertrophied (1.8 cm). There were multiple recent myocardial infarcts in the upper anterior septum and anterior lateral wall of the left venticle. The right coronary artery was sclerotic, but patent. There was severe sclerosis, with occlusion of the lumen, at the bifurcation of the left coronary artery. The valves were normal. A 3 × 2.5 × 1 cm soft, dark red, sessile globular nodule was attached to the wall of the left atrium in the region of fossa ovalis (Fig. 1). The cut surface of this nodule was soft, partially gelatinous, and red-brown. The remainder of the organs were congested, but otherwise appeared normal.

Histologically, the nodule contained a loose, sparsely cellular stroma containing spindle, polyhedral, and stellate cells. Occasional lymphocytes were scattered throughout. Capillaries lined by plump endothelial cells were present in the myxoid areas. At the site of attachment to the atrial surface, numerous prominent blood vessels penetrated the base of the nodule. Abundant free iron granules, hematoidin pigment, hemosiderin-laden macrophages and fibrous tissue were found at the base and central portion of the structure. Numerous aggregates of connective tissue fibers, encrusted with dark brown-blue pigment, were within this area (Fig. 2). Prussian blue and von Kossa stains revealed both iron and calcium in these structures. Foci of recent hemorrhage were noted near the surface of the nodule.

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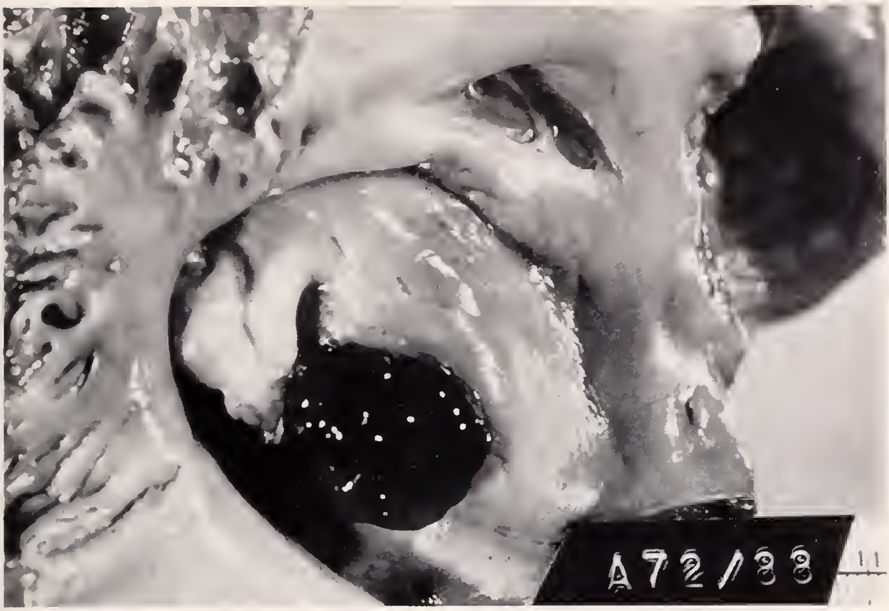

FIG. 1. Hemorrhagic myxoma attached to wall of left atrium adjacent to foramen ovale.

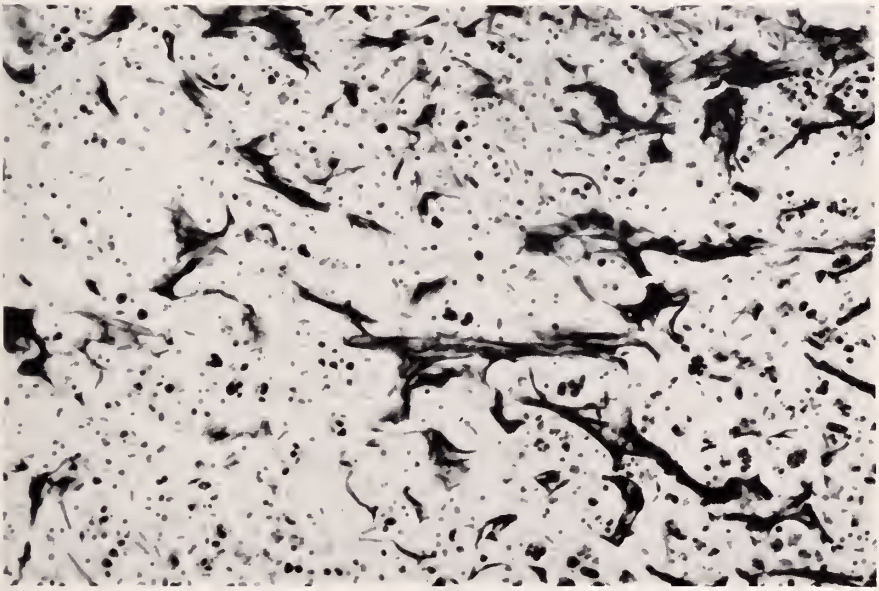

FIG. 2. Gamna-Gandy component of myxoma consisting of connective tissue fibers encrusted with iron and calcium salts (hematoxylin-eosin, original magnification 100×).

Comment

An isolated Gamna-Gandy nodule was recently described attached to the left atrial wall adjacent to the fossa ovalis (3). This was interpreted as organization of hemorrhage within the atrial wall. However, the atrial septum is a common site of cardiac myxoma. These tumors often show degenerative changes such as calcification, ossification, hemorrhage and fibrosis (4–6). Although the nature of cardiac myxoma is still controversial, most observers consider these structures to be neoplasms rather than an organized mural thrombus (7, 8).

The present case illustrates a Gamna-Gandy body within a myxoma of the left atrium. This association suggests that the formation of Gamna-Gandy bodies at this site in the heart represents an unusual degenerative change in a cardiac myxoma. A right atrial myxomatous tumor containing a similar siderotic structure has been previously reported (9).

Degenerative changes within a cardiac myxoma may occur spontaneously. In our case, however, other factors should also be considered, such as anoxia, hypotension, anticoagulant therapy, or trauma arising from the cardiac bypass procedure.

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Ischemic Necrosis of Bone in Sickle Cell Trait

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Abstract

While sickle cell trait is usually regarded as a benign condition, there is increasing evidence that complicating visceral and bone infarcts can occur. In order to assess the incidence of ischemic necrosis of bone in the presence of SA hemoglobin, all abnormal hemoglobin electrophoretic patterns encountered at the Hospital for Joint Diseases and Medical Center over a five-year period were reviewed. Patients with SA hemoglobin identified by hemoglobin electrophoresis were selected for further study. In addition, cases of bone involvement in sickle cell trait discovered during clinical work-up were included.

Four new cases of ischemic bone necrosis in sickle cell trait were added to seven found in a review of the literature. The new cases range from 11 years of age to 45. Three involve the hip primarily and the fourth represents a case of giant bone infarct of the ilium.

We conclude that ischemic changes, which have been well described in the bones of patients with SS and SC disease, may also be a significant problem in sickle cell trait.

Sickle cell trait, a heterozygous genetic variant of sickle cell anemia, is generally regarded as a benign condition. It exists in approximately eight percent of the American black population and is characterized by the presence of positive sickle preparation without anemia. The hemoglobin abnormality consists of the combination of S hemoglobin with variable amounts of A. The percentage of S hemoglobin ranges from 25 percent to slightly less than 50 percent.

Bone changes in sickle cell anemia (SS) are common and well documented (1–11). Likewise, bone changes occur frequently in the SC variant, particularly in relation to the hip joint. In sickle cell trait, on the other hand, there are few reported cases of bone necrosis (1, 2, 6, 7, 12, 13). These have been limited to the articular ends of long bones.

While sickle cell trait is usually regarded as an innocuous condition, there is increasing evidence that complicating visceral and bone infarcts can occur. In order to assess the incidence of ischemic necrosis of bone in the presence of SA hemoglobin, all abnormal hemoglobin electrophoretic patterns encountered at the Hospital for Joint Diseases and Medical Center over a five-year period were reviewed. There were a total of 95 cases with abnormal electrophoretic patterns. Of these, 69 had sickle hemoglobinopathies. Forty-one were examples of sickle cell trait (SA), 19 were homozygous sickle cell disease (SS), and nine were combinations of C hemoglobin and S hemoglobin (SC disease). Patients with SA hemoglobin identified by quantitative electrophoretic studies or by

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routine hemoglobin determinations greater than 13 gm percent were selected for further study.

In addition, cases of bone involvement in sickle cell trait discovered during clinical work-up at the Hospital for Joint Diseases and Medical Center and The Mount Sinai Hospital were included.

Four new cases of ischemic bone necrosis in sickle cell trait were added to seven found in a review of the literature. The new cases range from 11 years of age to 45. Three involve the hip primarily, and the fourth represents a case of giant bone infarct of the ilium.

We conclude that ischemic changes, which have been well described in the bones of patients with SS, SC, and S-thalassemia (1, 14, 15), may also be a significant problem in sickle cell trait. Quantitative hemoglobin electrophoresis is helpful to differentiate SA from mild sickle cell disease described by Steinberg et al as well as the double heterozygous states such as combinations of hemoglobin S with α or β thalassemia, S-D disease or S-hereditary persistence of fetal hemoglobin (16).

In this paper, the four additional cases of sickle cell trait with ischemic necrosis of bone are described, and the pertinent literature summarized.

Materials and Methods

Four cases of ischemic necrosis of bone occurring in patients with sickle cell trait (AS) were encountered in the patient population at the Hospital for Joint Diseases and Medical Center and The Mount Sinai Hospital from 1964–1972. Two of these (Cases 9, 10) were discovered in the course of a review of abnormal hemoglobin electrophoretic patterns at the Hospital for Joint Diseases and Medical Center, and two were diagnosed as having sickle cell trait during clinical work-ups for hip and back pain (Cases 8, 11). Three of the four new cases were operated upon and showed pathologic evidence of ischemic necrosis of bone. All of the new cases of SA hemoglobin reported here were further documented by means of a quantitative hemoglobin electrophoresis or an SA pattern and a hemoglobin level of greater than 13 gm percent. Other laboratory data consisted of hematocrit, sickle cell preparation, and bilirubin determinations. Roentgenograms of the hips and other bones were available in all cases.

Case Reports

Case 8. A 46-year-old black man was admitted to The Mount Sinai Hospital with a chief complaint of low back pain. He had first experienced back pain three years prior to admission, with an exacerbation noted two months prior to admission. There was no history of exposure to compressed air environment and no history of pancreatitis or alcoholism. Laboratory data included hemoglobin 12 gm, hematocrit 41 per cent, sickle cell preparation positive, bilirubin normal, hemoglobin electrophoresis SA with A_2 2.4 percent.

Physical examination was unremarkable.

Radiographic examination of the pelvis showed a heavily calcified lesion in the left ilium occupying most of the bone (Fig. 1A). The increase in density was confined to the ilium, and there was no expansion of the bone or extension into surrounding soft tissues.

Biopsy, open and needle, performed in several areas of the iliac lesion, (Fig. 1B) revealed bone necrosis.

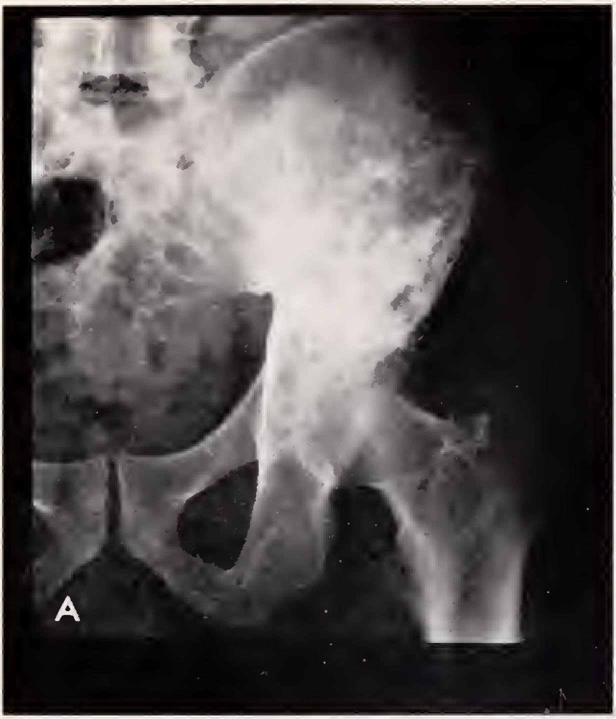

FIG. 1A. *Case 8.* A large bone infarct in a patient with sickle cell trait. Roentgenogram of left ilium showing a heavily calcified lesion extending from the midportion of the bone to the acetabulum. No expansion of the bone or extension to the soft tissue. The hip joint was not affected and the remaining bones were normal in appearance.

Case 9. An 11-year-old black girl presented with a complaint of pain in the right hip. She had a history of four years' non-weight-bearing treatment for Perthes disease of the right hip. She had no clinical anemia. Laboratory data included hemoglobin of 13 gm, hematocrit 40 percent, sickle cell preparation positive, and electrophoresis pattern of SA.

She was treated conservatively, and by February 1972 considered herself symptom-free. Her right hip showed limited flexion and abduction.

Roentgenograms revealed changes interpreted as old Perthes disease of the right femoral head (Fig. 2) and a questionable deformity of the right capital humeral epiphysis and cystic changes in the right ulna.

Case 10. A 41-year-old black woman presented at the Hospital for Joint Diseases with a complaint of right hip and knee pain of 10 months' duration. Hemoglobin was 11.9 gm, hematocrit 37.1 percent, sickle cell preparation positive. Hemoglobin electrophoresis revealed S 35.8 percent, A 60.8 percent, A_2 3.3 percent, F 1.1 percent.

Physical examination revealed an antalgic gait with mild restriction of motion.

Roentgenograms revealed narrowing of the joint space with an irregular and flattened surface of the femoral head and subchondral sclerosis and rarefaction compatible with aseptic necrosis (Fig. 3A). She was treated with a McMurray osteotomy of the right femoral head in 1961, followed by Austin Moore prosthesis in 1966 (Fig. 3B). The femoral head showed aseptic necrosis and secondary degenerative joint disease. Sickled erythrocytes were found in the lumens of blood vessels (Figs. 3C, D). At the present time, she is walking with pain, but without crutches.

Case 11. A 45-year-old black man presented with a two-year history of pain in both hips, the left greater than the right. There was also a history of moderate alcohol intake.

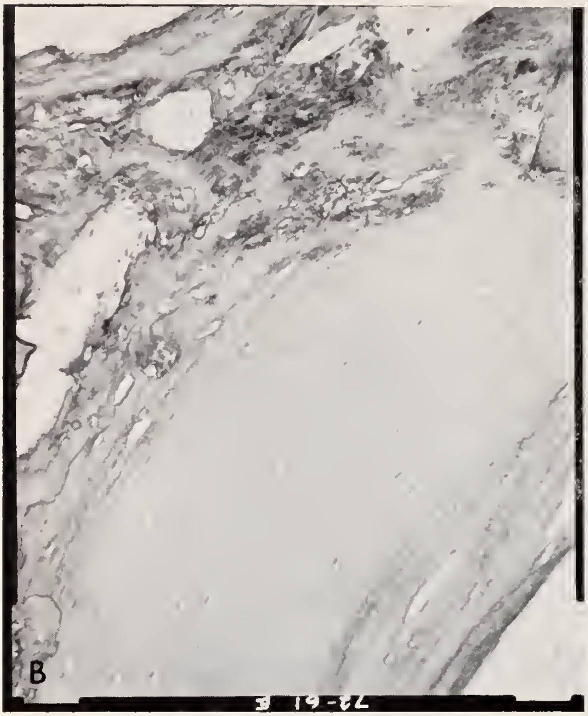

Fig. 1B. *Case 8.* Photomicrograph of bone removed by needle biopsy from area of increased density in left ilium. There is necrosis of lamellar bone with lacunae devoid of steocytes and calcific deposits in the hyalinized collagenous tissue of the intervening stroma. These changes are consistent with a bone infarct (Hematoxylin and eosin × 320).

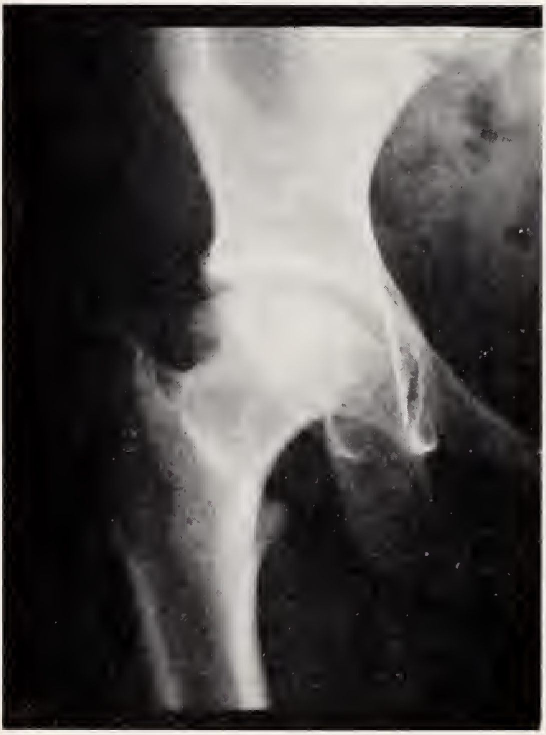

Fig. 2. *Case 9.* An 11-year-old girl with sickle cell trait. Roentgenogram of right hip showing flattened contour of femoral head, subchondral sclerosis, and mottled radiolucency. These changes had been interpreted as old Perthes disease of the femoral head. Other roentgenologic abnormalities were noted in the right capital humeral epiphysis and right ulna.

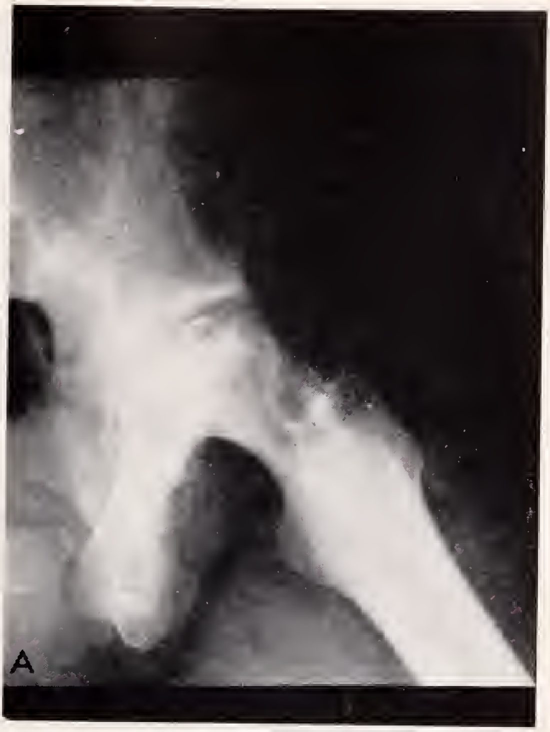

Fig. 3A. *Case 10.* A forty-one-year-old woman with sickle cell trait and aseptic necrosis of right hip. Roentgenogram taken in 1961 showing narrowing of joint space and irregular contour of femoral head due to ischemic necrosis and collapse of subchondral bone. Note cleft-like zone of radiolucency of the lateral aspect.

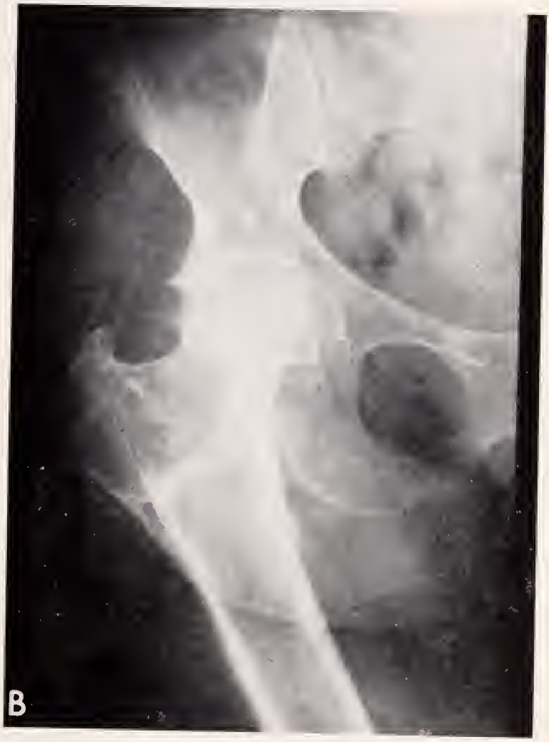

Fig. 3B. *Case 10.* Roentgenogram of right hip in 1964, one year after intertrochanteric osteotomy. There is further distortion of the femoral head with changes of secondary degenerative joint disease. Marginal osteophytes are present both on the acetabular rim and the femoral head and there are areas of subchondral radiolucency as well as sclerosis.

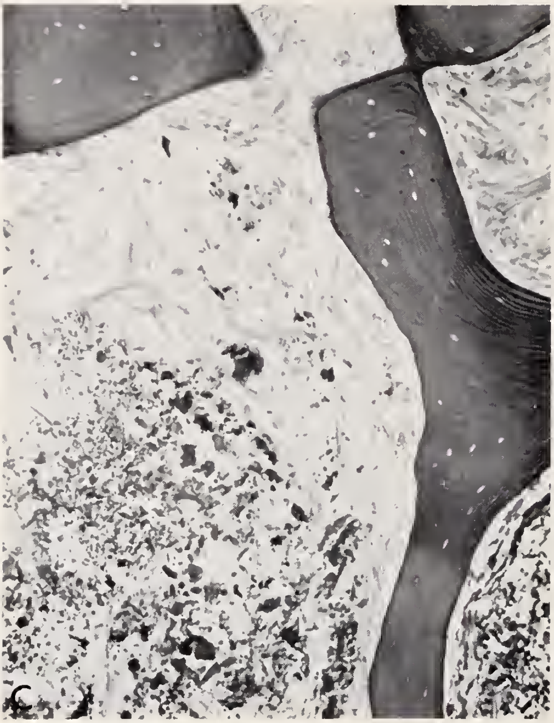

FIG. 3C. *Case 10.* Photomicrograph of subchondral bone from femoral head removed in 1966. There is ischemic necrosis of cancellous bone trabeculae, fibrous scarring of the intertrabecular spaces, and calcific detritus (Hematoxylin and eosin × 120).

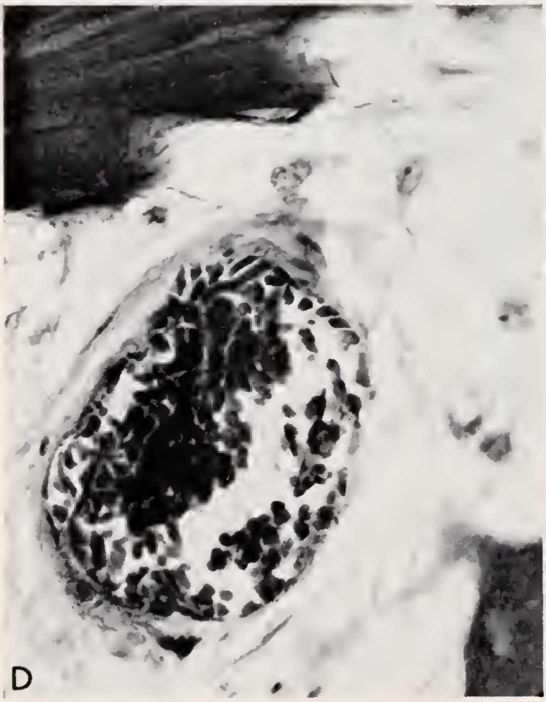

FIG. 3D. *Case 10.* Photomicrograph of subchondral bone adjacent to area of ischemic necrosis showing sickled erythrocytes within the lumen of a blood vessel (Hematoxylin and eosin × 800).

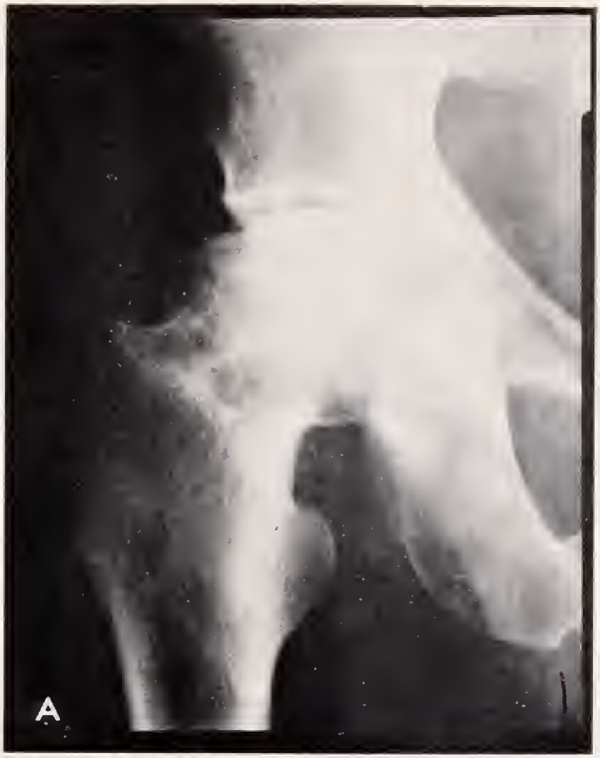

FIG. 4A. *Case 11.* A forty-five-year-old man with bilateral ischemic necrosis of the femoral heads and sickle cell trait. Roentgenogram of the left hip showing an area of increased density and abnormal contour of the femoral head.

Physical examination revealed moderate limitation of motion of both hips. Hemoglobin was 16.3 gm, hematocrit 46.6 percent, sickle cell preparation positive, hemoglobin electrophoresis SA, quantitative S 31.1 percent, A_1 57.7 percent, A_2 4.9 percent, F 3.6 percent.

Roentgenograms showed depression of the cortex of the weight-bearing portions of the left and right femoral heads characteristic of ischemic necrosis (Fig. 4A). In addition both humeral heads showed roentgenographic evidence of aseptic necrosis.

He underwent a low friction arthroplasty of the left hip. The femoral head showed a cleft in the necrotic subchondral bone with a sharply outlined border to the infarcted zone (Fig. 4B).

Discussion

The incidence of bone infarction associated with hemoglobinopathies is greatest in the SC variant (20–60 percent) (1). Its occurrence is well established in sickle cell anemia (SS) where it most frequently involves the hip (4–12 percent) (1). Although visceral infarcts and sudden death have been reported in sickle cell trait (16), a review of the literature revealed only seven previously reported cases of ischemic necrosis of bone in this condition. It has also been rarely observed in the S-thalassemia and SF genetic variants.

The age range of patients with sickle cell trait and bone infarction is 11–48

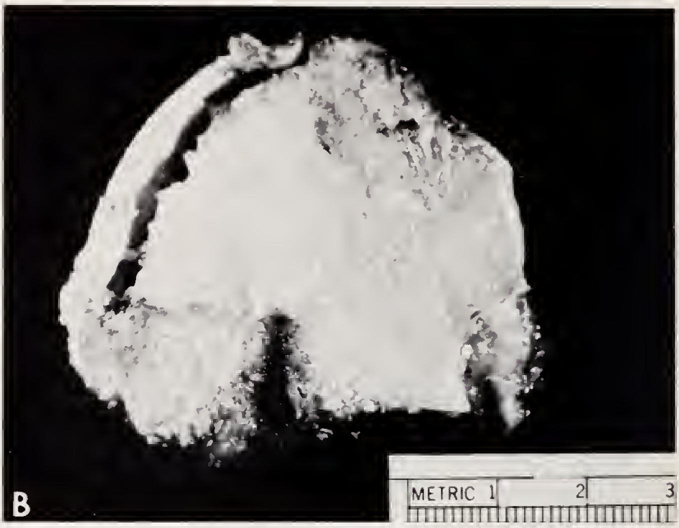

Fig. 4B. *Case 10.* Gross photograph of the bisected left femoral head removed at surgery. Note the intact articular cartilage and the cleft in the subchondral bone. The pale necrotic area is sharply demarcated.

years (Table I). There were seven males and four females. At least one femoral head was involved in ten cases. Four patients also had ischemic changes in the humeral heads. In one of the author's cases there was a large infarct occupying the left iliac wing. Pathologic documentation was obtained in three cases of the present series and two of the previously reported group. The histologic changes were indistinguishable from bone ischemic necrosis of other causes, except for the presence of sickled erythrocytes in blood vessels (Fig. 3D).

All patients in the group reported here had SA hemoglobin demonstrated by quantitative hemoglobin electrophoresis (Cases 8, 10, 11) or had qualitative studies revealing SA hemoglobin and no anemia (hemoglobin greater than 13 gm). In none of the previously reported cases is it specified that S-thalassemia hemoglobinopathy has been excluded by quantitation of the A and F hemoglobin fractions, though some authors indicate the need for this distinction (1, 2). S-thalassemia is characterized by increased levels of A_2 and F hemoglobin in addition to S hemoglobin. The S hemoglobin fraction is also higher in S-thalassemia than in sickle cell trait, and, in these patients, anemia is usually noted. Target cells are usually noted in patients with S-thalassemia.

In the new cases reported here, other underlying causes of bone infarction, such as trauma, alcoholism, pancreatitis, decompression disease, systemic lupus erythematosus, and steroid therapy, have been excluded on the basis of history and appropriate laboratory tests. None of the new cases had visceral infarcts.

Idiopathic ischemic necrosis is rarely reported in adult blacks. A review of the literature reveals only three cases, specifically cited as occurring in black patients (18). Perthes disease, too, is seldom reported in black children. Trueta

TABLE 1

Cases of Ischemic Bone Necrosis in Sickle Cell Trait

Case #	Age	Sex	Race	Presenting Complaint	Hgv	Hct	Sickle Prep	Hemoglobin Electrophoresis	Bilirubin
1	38	M	Black	Pain in both hips and left shoulder	13-14 gm	—	Positive	—	0.3 mgm indirect
2	25	M	Black	Rt knee pain and migratory arthralgias	normal	—	Positive	20% S, 80% A	—
3	48	M	Black	Rt hip and knee pain	15.2 gm	45	Positive	SA	normal
4	41	M	Black	Rt hip pain	15.3 gm	57	Positive	SA	—
5	43	M	Black	Back pain	14.9 gm	42.5	—	SA	—
6	41	F	Black	Rt hip and knee pain	—	—	—	"proved sickle cell trait"	—
7	24	F	Black	Left hip pain	12.5 gm	—	Positive	SA	—
8	41	M	Black	Back pain	13.5 gm	41	Positive	SA (2.4% A_2)	normal
9	11	F	Black	Rt hip pain	13.0 gm	40	Positive	SA	—
10	41	F	Black	Rt hip and knee pain	9–12.3 gm	29–40	Positive	SA (3.3% A_2)	—
11	45	M	Black	Pain Both hips	16.3 gm	46.6	Positive	SA (4.9% A_2)	—

Case #	Distribution of Lesions	Treatment	Pathology	
1	Both femoral heads, both humeral heads	No surgery		Moseley & Manly (1953)
2	Sacroiliac joints, femoral and humeral heads, sub. talar joints, rt knee periosteal elevation in tibias and fibulas	No surgery		Stewart (1960)
3	Right hip, distal, right femur, right humeral head, left elbow	Curettement and packing head and neck of rt femur	Necrotic bone, no evidence of infection	Ratcliff & Wolf (1962)
4	Right femoral head	Curettement femoral head	Necrotic bone, no evidence of infection	Ratcliff & Wolf (1962)
5	Left femoral head, right femoral head	No surgery		Blau & Hamerman (1967)
6	Right Hip	No surgery		Haddad (1967)
7	Left Hip	No surgery		Chung & Ralston (1969)
8	Left ilium	Biopsy	Bone infarct	Nachamie & Dorfman (1973)
9	Rt hip ? humeral head and ulna	No surgery		Nachamie & Dorfman (1973)
10	Right femoral head	Austin-Moore prosthesis, right	Ischemic necrosis of femoral head	Nachamie & Dorfman (1973)
11	Right and left femoral head, both humeral heads	Charnley low friction arthroplasty, left	Ischemic necrosis of femoral head	Nachamie & Dorfman (1973)

(19) mentions a three percent incidence in Brazilian hospitals having a more than 50 percent black population. It is not stated whether any of the patients in that study had SS, SC or SA hemoglobin. Trueta attributes this low incidence to a persistence of the ligamentum teres artery in blacks. The presence of sickle cell trait appears to be the only possible contributing factor in the four patients reported here. Such instances of bone necrosis in sickle cell trait may be more common than is generally realized. Even when anemia is not present, the occurrence of ischemic necrosis of bone in black patients should arouse suspicion of sickle cell trait.

Acknowledgment

We gratefully acknowledge the help of Drs. Herman Robbins and Aaron Gold in allowing us to use cases 11 and 12.

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Stereoradiography of the Posterior Fossa with Standard Angiogram Equipment

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Introduction

Stereoscopy has long been considered a valuable adjunct to cerebral angiography for three-dimensional study of the vascular detail of intracranial lesions (1). The toxicity of earlier contrast media (2) and the necessity of multiple injections (3) stimulated the introduction of biplane operation (4), stereoscopic serial angiography with double tubes (5), and even serial simultaneous biplane stereoscopic arteriography with two shifting tubes (6) or two sets of double tubes (7). Adequate opacification of the intrinsic vasculature of the posterior fossa was only possible by direct vertebral puncture (8) or catheterization (9) until the description of bilateral simultaneous retrograde brachial angiography (10), but two mechanical injectors were required. Many hospitals are without benefit of elaborate x-ray apparatus or more than one pressure injector. The evolved method which follows employs only standard angiogram equipment and produces satisfactory stereoscopic views of the blood vessels of the cerebellum, brainstem and occipital lobes.

Materials and Methods

The patients ordinarily received phenobarbital 100 mg and atropine 0.4 mg intramuscularly as premedication. After sterile preparation of the skin and local infiltration with lidocaine, both brachial arteries were cannulated with #16 Cournand needles, which were subsequently attached to 30-inch lengths of tubing. Needle placement was checked by scout films of successive hand injections of 5 cc of meglumine iothalamate 60%. A three-way connector was utilized to connect the two sides to the automatic injector. One extra length of tubing was inserted between the "Y" connector and the needle in the left brachial artery to compensate for the shorter anatomical access to the vertebral artery from the left brachium (Fig. 1).

Before each injection of meglumine iothalamate, the entire tubing arrangement contained only citrated saline. Flushing the right and left sides individually was accomplished with two two-way valves. The chamber of the injector was filled to 80 cc with contrast medium, and the x-ray equipment set to begin the exposure of serial films at 20 cc remaining. An operating pressure of 6.5 Kg/cm^2 and an injection rate of 60 cc/sec were used. Each series of films was exposed at the rate of two per second for the first three seconds, and one per second for the next six seconds.

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Stereoscopic exposures were obtained by appropriately shifting the x-ray tube between the two injections for the anteroposterior views (Fig. 2) and similarly for the lateral views (Fig. 3). Once the patient's head had been taped in position, a vertical tube shift equal to ten percent of the target-film distance pro-

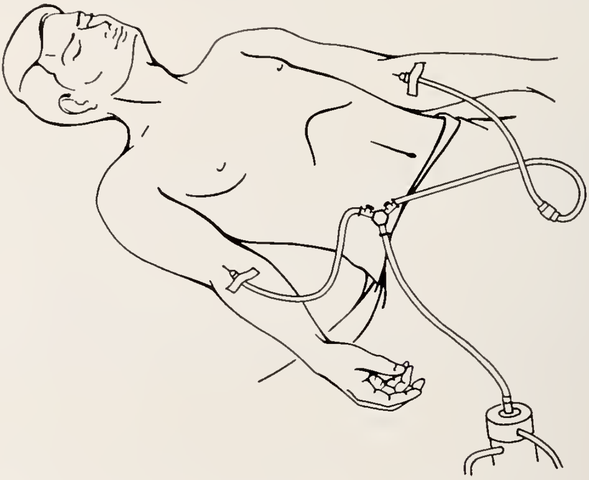

FIG. 1. Drawing illustrating modified "Y" tubing arrangement.

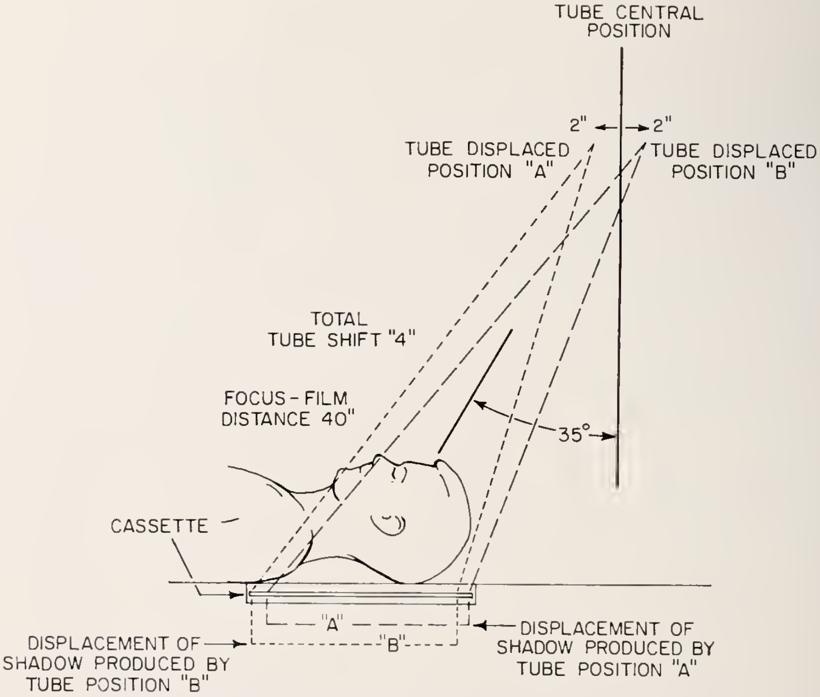
TUBE CENTRAL POSITION
2"
2"
TUBE DISPLACED POSITION "A"
TUBE DISPLACED POSITION "B"
TOTAL TUBE SHIFT "4"
FOCUS-FILM DISTANCE 40"
35°
CASSETTE
"A"
"B"
DISPLACEMENT OF SHADOW PRODUCED BY TUBE POSITION "B"
DISPLACEMENT OF SHADOW PRODUCED BY TUBE POSITION "A"

FIG. 2. Stereoscopic technique for anteroposterior view.

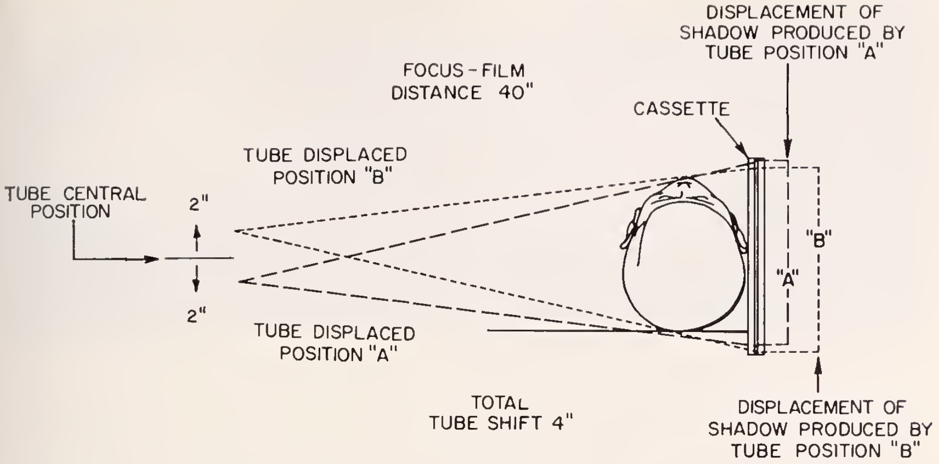
DISPLACEMENT OF SHADOW PRODUCED BY TUBE POSITION "A"
FOCUS - FILM DISTANCE 40"
CASSETTE
TUBE DISPLACED POSITION "B"
TUBE CENTRAL POSITION
2"
2"
"B"
"A"
TUBE DISPLACED POSITION "A"
TOTAL TUBE SHIFT 4"
DISPLACEMENT OF SHADOW PRODUCED BY TUBE POSITION "B"

Fig. 3. Stereoscopic technique for lateral view.

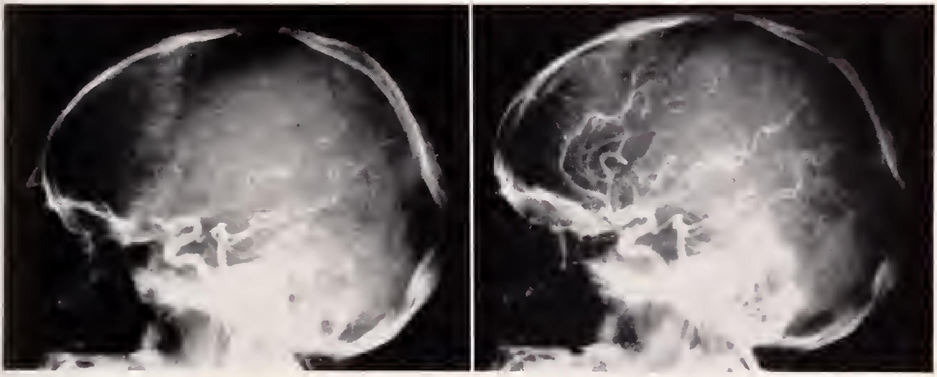

Fig. 4. Lateral projection. Left: tube position "A" for left eye film. Right: tube position "B" for right eye film.

duced satisfactory stereoscopic pairs in the lateral projection (Fig. 4). In the Towne projection, a horizontal tube shift in the direction of the long axis of the grid provided the same effect (Fig. 5).

Four separate injections were regularly performed; the two Towne projections required right carotid compression during the arterial phase to eliminate visualization and overlap of the anterior circulation. The tubing and connector and valves constituted 20 cc of dead space; between injections, back-bleeding eliminated the residual meglumine iothalamate. Including the scout films, the total contrast material delivered to the patient was 250 cc.

Stereoscopic films are best examined at length with a suitable stereoscope. Review is possible without special optical equipment by cross-eyed stereoscopy (Fig. 6). The dissociation of accommodation and convergence can be mastered by most physicians with practice.

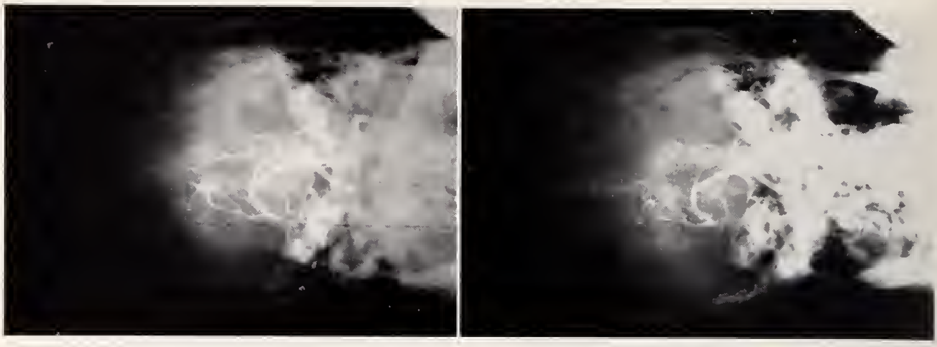

FIG. 5. Towne projection. Left: tube position "A" for left eye film. Right: tube position "B" for right eye film.

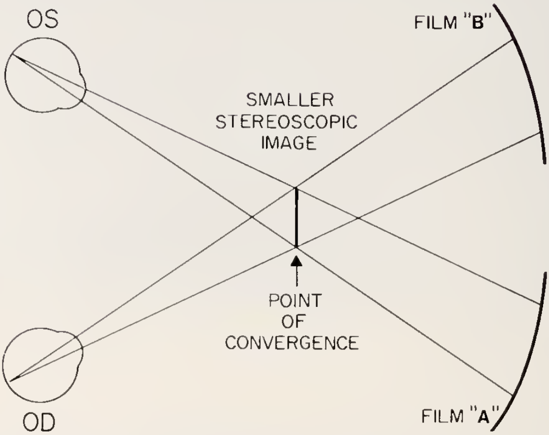

FIG. 6. Cross-eyed stereoscopy: converging on a point considerably in front while still accommodating to the plane of the films.

Results

Twenty cases of posterior fossa dysfunction underwent stereoangiography during a one-year period. Twelve patients were in good clinical condition, five were stabilized on steroids, and three were moribund. In our hands, there were no complications directly related to the cannulation of both brachial arteries, the utilization of "Y" tubing, four successive mechanical injections, or the relatively large amount of contrast medium.

Discussion

Compensation for the anatomical differences in the aortic arch has only been achieved previously during bilateral brachial angiography by recourse to two pressure injectors with a quarter-second delay on the left side (10). Because of the necessity of staggering the injections, a single injector with a "Y" tube was considered inadequate to prevent some dilution of the contrast material by unopacified blood from the right side. With the modified arrangement employing longer tubing on the left side, meglumine iothalamate can be observed flowing synchronously up both sides of the neck and meeting at the vertebrobasilar junction.

Concern over reduced blood supply to the brainstem caused by contrast medium entering both vertebral arteries simultaneously and repeatedly does not seem warranted. In the presence of a hypoplastic right vertebral artery, left brachial injection carried no greater risk (10). Unlike carotid injection, most of the meglumine iothalamate injected into the brachial arteries is lost to the corporal circulation.

Ideally, both films of a stereoscopic pair should visualize exactly the same structures. With a standardized injection technique and exposure rate, the left eye film and the right eye film still differ somewhat in the filling of the cerebral vasculature (Fig. 4, Fig. 5). The dissimilarity is even greater in the paired films produced by stereoscopic serial angiography (11). Review of the entire set of exposures provides segmental but virtually complete three-dimensional views of the blood vessels of the brainstem, cerebellum, and occipital lobes.

Conclusions

In the diagnostic evaluation of patients with neurologic symptoms and signs referable to the posterior fossa, cerebral angiography has become the initial neuroradiologic procedure of choice (12). Bilateral simultaneous retrograde brachial arteriograms provide excellent visualization of the intrinsic vasculature of the cerebellum, brainstem, and occipital lobes. Stereoscopic technique facilitates the vessel-to-vessel correlation essential to the differentiation of fourth ventricle tumors from brainstem tumors. Stereoangiography of the posterior fossa may be readily accomplished with x-ray equipment commonly available.

Acknowledgment

The authors wish to express their thanks to Dr. Yun Peng Huang, whose works and teaching stimulated this project.

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Hemophilus Parainfluenzae Meningitis: A Case in an Adult

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Abstract

Hemophilus parainfluenzae was isolated from the cerebrospinal fluid of a 16 year-old girl in the second trimester of pregnancy who had meningitis and a petechial rash. The clinical course was complicated by transient lower gastrointestinal and urinary tract bleeding, and threatened abortion, but recovery with ampicillin therapy was complete and a normal female infant was subsequently born. Although H. parainfluenzae has been reported in 9 cases of childhood meningitis under the age of 5, this is the first adult case to our knowledge.

Hemophilus parainfluenzae has recently been recognized as a cause of bacterial meningitis in children (1–3). We report what we believe is the first case of H. parainfluenzae meningitis in an adult—a primiparous 16-year-old girl with an unusual clinical picture resembling meningococcal disease.

Case Report

A previously healthy 16-year-old white girl in the second trimester of pregnancy was admitted to the City Hospital Center at Elmhurst on February 16, 1968, febrile and semicomatose. On the evening prior to admission she had a shaking chill followed by fever and malaise. The next morning nausea, vomiting, and a rash were present. Throughout the day she became progressively more stuporous with periods of irritability. By evening she was unresponsive and was brought to the emergency room.

Physical examination revealed an acutely and severely ill semicomatose girl who showed marked irritability when disturbed. The rectal temperature was 39.9°C., blood pressure 125/60 mm Hg., pulse 120/minute and regular, and the respiratory rate 30/minute. Petechiae were present over the entire body but were most prominent on the extremities. The pupils were slightly dilated but responsive to light, the conjunctivae injected, and the fundi normal. Except for a sinus tachycardia, examination of the cardiovascular system and chest was normal. Abdominal examination was compatible with a sixteen week intrauterine pregnancy. The cervix was closed and there was no discharge, bleeding, or evidence of instrumentation. Sensation and motor strength could not be completely evaluated, but movements were symmetrical and well preserved. Although the neck was supple, Kernig's and Brudzinski's signs were present. The deep tendon reflexes were equal. The plantar response was flexion.

Lumbar puncture obtained grossly cloudy cerebrospinal fluid with a cell count of greater than 10,000 WBC's per cm, all of which were polymorphonuclear granulocytes. The protein was 405 mgm %; the glucose too low to be determined. Manometric pressures could not be accurately obtained because of the patient's inability to cooperate. No organisms were seen on smear. Cultures grew a Hemophilus species identified on the basis of a requirement for V factor, but not X factor, under aerobic conditions, and absence of hemolysin production, as Hemophilus parainfluenzae. The isolate was sensitive to ampicillin, chloramphenicol, strep-

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tomycin, and cephalothin but resistant to erythromycin, penicillin, albamycin, and oxacillin. Smears of petechial blood were made and found to contain gram negative bacilli. Three blood cultures were sterile. One of two throat cultures grew Hemophilus parainfluenzae sensitive to penicillin, erythromycin, tetracycline, neomycin, and albamycin in addition to those antibiotics to which the CSF isolate was sensitive. Cultures of the nasopharynx and cervix uteri failed to grow the pathogen.

Other pertinent laboratory findings included a hemoglobin of 11.5 grams %, packed cell volume 35%, white blood cell count 25 500 per cmm with a differential of 52% segmented neutrophils, 44% bands, and 3% lymphocytes, and platelets 184,000 per cmm. A prothrombin time of 34 seconds with a 12 second control was initially reported, but an immediately repeated sample was 16/12 seconds. Urinalysis showed a faint trace of albumin, trace glucose, and 3—5 WBC's and 12—15 RBC's per high power field of sediment. Serum glucose was 136 mgm %, serum potassium 2.5 mEq/L, sodium 148 mEq/L, chloride 104 mEq/L, and carbon dioxide 14 mEq/L. Arterial blood gases were pH 7.34, pCO_2 17 mm Hg., and pO_2 114 mm Hg. The blood urea nitrogen and recumbent chest X-ray were normal.

Treatment was initiated with intravenous sodium ampicillin, potassium penicillin G, hydrocortisone, sodium amytal for sedation, and fluids. The immediate hospital course was complicated by several events. Uterine contractions began within the first few hours and abortion was thought to be inevitable but contractions stopped by the morning after admission. Concurrently a small amount of blood was noted in an indwelling Foley catheter, and a small amount of bright red blood was passed per rectum. A gastric aspiration for blood was negative. At this time the platelet count was 184,000 per cmm, fibrinogen 360 mgm%, prothrombin time again 16/12 seconds, but the packed cell volume was now 25% and two units of packed red cells were administered slowly. Bleeding ceased and by hospital day 3 the patient was responsive with a temperature of 37.8°C. The neck was now noted to be stiff. A compensated respiratory alkalosis and metabolic acidosis was thought to be present with the serum CO_2 16 mEq/l, arterial pH 7.42, and pCO_2 22 mm Hg. The prothrombin time remained at 16/12 seconds, the haptoglobin was 206 mgm %, and the total bilirubin 1.9 mgm %. Clinical improvement was rapid with fading of the petechial rash and complete lysis of fever. Lumbar puncture was repeated on hospital day 6 and revealed an elevated opening pressure of 440 mm water, protein 30 mgm %, sugar 32 mgm %, 45 cells per cmm—all lymphocytes—and no growth on culture. The fundi were normal and signs and symptoms of increased intracranial pressure were absent. Lumbar puncture performed 11 days after admission showed an opening pressure of 160 mm water with 25 mgm % protein, 50 mgm % glucose, no cells, and no growth on culture. The white blood count was now 14,900 per cmm with a differential count of 87% polymorphonuclear neutrophils, 1 band, 9 lymphocytes, and 3 monocytes. The hemoglobin was 13 gms. % and the packed cell volume 39%. Urticaria appeared and resolved with oral diphenylhydramine and discontinuance of ampicillin on hospital day 14. Penicillin G had been stopped when sensitivity studies became available. The prothrombin time remained elevated at 14/12.5 seconds. The total bilirubin was 0.7 mgm %. A glucose tolerance test performed after intravenous fluids were discontinued was normal. Studies to uncover a predisposing susceptibility to meningitis included a serum electrophoresis which revealed a low total protein, 5.8 grams % (normal 6.5 to 8.5 gms. %); low albumin, 2.8 gms. % (normal 3.5 to 5.5 gms. %) and elevated alpha 2 globulin, 1.0 gms. % (normal 0.5 to 0.9 gms. %). Quantitative immunoglobulin studies showed normal levels of IgG and IgA but an elevation of IgM to 140 mgm % (normal 88 ± 22 mgm %). A Sia-Wu water test was positive. Skull x-rays were normal. The remainder of the patient's hospital course was uneventful and the patient was discharged on March 4th with no neurologic sequelae. She was followed in the outpatient department and on June 30th, 1968, delivered a normal female infant.

Discussion

Bacilli of the genus Hemophilus are pleomorphic gram negative, non-motile, aerobic organisms which are further classified on the basis of their ability to hemolyze blood and a nutritional requirement for one or both of two factors present in blood: X and V factors (4, 5). Hemophilus parainfluenzae was first named by Rivers in 1922 who distinguished it as a Hemophilus species requiring only a heat-labile substance in unautoclaved yeast extracts (6). This substance, V factor, was subsequently shown to be nicotinamide adenine dinucleotide (NAD) (7). NADP and nicotinamide nucleoside may also function as V factor (7, 8). Hemophilus influenzae on the other hand requires not only V factor, but also a heat-stable factor in blood, iron protoporphyrin, termed X factor (4, 5, 9). X factor dependent species appear to have lost enzymatic activity responsible for synthesis of protoporphyrin from δ-amino levulinic acid (10). The growth of satellite colonies of Hemophilus species around colonies of other bacteria first described in 1897 (11) can also be utilized in elucidating their V and X factor requirements (12). Since V factor is synthesized by staphylococci and X factor is present in blood or chocolate agar, Hemophilus species may be classified by comparing their growth on blood and nutrient agar, under aerobic conditions, close to and distant from streaked staphylococcal colonies, as was done in this case. An X and V factor requirement is revealed by growth near the staphylococcal streak on blood agar only, while a V factor requirement shows growth near the staphylococcal streak on both blood and nutrient agar.

Hemophilus parainfluenzae is a well documented although infrequent cause of bacterial endocarditis (13–16). As Craven et al have noted, some cases of endocarditis caused by Hemophilus species have had clinical signs or autopsy findings of meningitis (14). These have been late in the clinical course and appear secondary to emboli in some cases, but in at least a few cases a generalized purulent leptomeningitis has been seen. However, primary bacterial meningitis caused by Hemophilus parainfluenzae has only recently been described in children (1–3), and we believe this report to be the first documented case in an adult. The first reported case was that of a two-day-old boy who recovered (1), as did a second patient, a 4-year-old boy (2), and seven additional cases (3). The report of Hable and her colleagues suggests that more widespread determination of the X and V factor requirements of Hemophilus isolates might reveal a considerably higher incidence of H. parainfluenzae than is now appreciated (3). Of 16 patients with Hemophilus infections (10 with meningitis), 10 patients with H. parainfluenzae were seen. Seven of the 10 cases of meningitis, ranging in age from 6 months to 4 years, were caused by this organism. In addition the organism was isolated from three patients who did not have meningitis: a patient with subacute bacterial endocarditis; a 60-year-old woman with chronic lymphocytic leukemia, hypogammaglobulinemia, H. parainfluenzae pneumonia and septicemia; and an 11-year-old girl with congenital tricuspid atresia and dental and brain abscesses. The only fatality was in the patient with chronic lymphocytic leukemia and sepsis (3). Organisms in the cases of Barnshaw and Phillips, and Hable et al were all sensitive to ampicillin, while Gullekson and Dumoff do not mention sensitivity studies with ampicillin (1–3). Organism sensitivity to penicillin varies in these reports and the oropharyngeal isolate from our patient was sensitive to penicillin as well as several other antibiotics to which the CSF isolate was not.

Our patient was initially thought to have meningococcal meningitis because of the petechial rash. However, petechiae have been reported in nonmeningococcal bacterial meningitis including those due to H. influenzae (17–23). One case with H. influenzae and one with pneumococcal meningitis in the Seattle group's experience had petechiae (17). In the case reported by Warthim, H. influenzae was cultured from a petechia (23). In a series of 120 patients with H. influenzal meningitis studies by Hsü-Ming and Tsai-Tao, two had localized skin lesions in the form of petechiae (22). The presence of gram negative bacilli in the petechial smear of our patient alerted the bacteriology laboratory to the presence of an unusual organism.

Meningitis is not thought to be more frequent during pregnancy (24), although cases near term have been reported (24, 25). Pregnancy is known to predispose experimental animals to the generalized Schwartzman reaction and to its possible clinical counterpart, endotoxin shock in man (26). Moreover, Hemophilus infections are frequently seen in veterinary medical practice (27), and H. parainfluenzae has been associated with the generalized Schwartzman reaction in pigs (28). Although the absence of renal impairment, confluent purpura, cardiovascular collapse, thrombocytopenia, or worsening on corticosteroid administration made a generalized Schwartzman reaction in our patient unlikely, the presence of hematuria, rectal bleeding and threatened abortion suggested an abnormal coagulation state. Detailed coagulation studies were not performed. Since disseminated intravascular coagulation has been seen in H. influenzal meningitis (29), and fibrinogen levels are known to increase throughout pregnancy, substantially in the second trimester (26, 30), it is tempting to speculate that the presence of a normal fibrinogen might represent a fall from an elevated level. Our patient's value of 360 mgm % was within normal limits for the second trimester and probably did not represent a marked fall. Together with a normal platelet count on several occasions this made less likely the presence of significant DIC. Fibrin split products were not measured, however, and we could not rule out some degree of fibrinolysis.

A predisposing cause for meningitis could not be found in our patient. There was neither historical nor objective evidence of head trauma or of recent infection in sinuses or mastoid air cells. IgA and IgG levels were normal. The Sia-Wu euglobulin test was said to be positive and the serum IgM was slightly elevated to 140 mgm %. A positive Sia water test has been reported in polyclonal as well as monoclonal gammopathies and in a significant number of normal or hypogammaglobulinemic sera (31). Moreover, the pH of the water used is of considerable importance (32). The significance of this finding in our patient is not clear but it is conceivable that it represents an increase of euglobulin in response to her infection.

H. parainfluenzae appears to be more prevalent than is usually recognized. Parker and Hoeprich studied Hemophilus species isolated from 106 well babies and a selection of 1211 throat swabs from patients of all ages. H. parainfluenzae was predominant in throat specimens from both groups, although in the babies' nasopharynges H. influenzae was usually found. Only 22 isolates of Hemophilus species among 319 specimens from patients over 13 years of age were found, but these were predominately H. parainfluenzae (33). Along with the experience of Hable et al (3) this strongly argues the advisability of determining the X and V factor requirements of Hemophilus isolates from patients with bacterial meningitis. The absence of mortality among the ten cases reported thus far is noteworthy but it remains to be seen if infection with H. parainfluenzae carries a more favorable prognosis than infection with H. influenzae. In all cases in which sensitivity to ampicillin was tested, the cerebrospinal fluid isolates of H. parainfluenzae have been sensitive and use of ampicillin pending the results of sensitivity studies appears to be warranted.

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Perforated Cystic Leiomyoma of the Ileum: Report of a Case

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Introduction

Abdominal pain and/or lower gastrointestinal bleeding are the usual presenting symptoms of small intestinal leiomyomas. A cystic leiomyoma of the ileum presenting as a perforated viscus is extremely rare.

Case Report

An 86-year-old white woman was admitted to The Mount Sinai Hospital because of rectal bleeding of one week's duration.

She complained of mild abdominal pain and had had occasional episodes of vomiting and bowel movements with dark red blood in the past week.

Two years prior to admission the patient had been admitted for a similar problem. During the work-up a sigmoid perforation occurred for which a transverse colostomy was performed. The bleeding ceased spontaneously. An upper gastrointestinal series and a barium enema were interpreted as normal. The source of bleeding was not determined. The colostomy was closed one year prior to admission.

On admission the patient's temperature was 101°F; blood pressure—160/90; respirations—16/min. Her abdomen was mildly tender throughout. Guarding, rigidity and rebound were absent. There were no masses palpable. Rectal examination was negative except for the presence of dark burgundy red blood on the examining finger.

Hemoglobin was 6.4 gms%; white blood count was 14,000 with 76% polymorphoneutrophils.

Blood transfusions were given and no further bleeding was noted. One day after admission she developed generalized abdominal tenderness with guarding and rigidity and her temperature rose to 103.4°F. The tenderness seemed to be more localized to the right lower quadrant of the abdomen. The patient was operated upon with the presumptive diagnosis of acute appendicitis or diverticulitis.

The abdomen was entered through a lower midline incision. Cloudy fluid and exudate were present. In the right pelvis, a perforated cystic tumor of the ileum was found. The tumor measured 11.5 cm in diameter. Resection of the ileum was performed with a 10 cm margin on each side of the tumor. The serosa of the tumor was covered with a yellow fibrinous material and the lumen was filled with a grayish-pink, hemorrhagic gelatinous tissue. Pathological report was "perforated benign leiomyoma of the ileum" (Fig. 1). Cystic degeneration and ulceration of the overlying mucosa were apparent in the gross specimen.

Postoperatively, the patient developed a wound infection which responded to antibiotics. Mild upper gastrointestinal bleeding secondary to a prepyloric ulcer responded to medical management. The patient is now 24 months postoperative and has remained asymptomatic.

Discussion

Leiomyomas are found throughout the small intestine and account for about 20% of all small intestinal tumors. Approximately 25–35% are located in the duodenum; 35–50% in the jejunum; 30–50% in the ileum; and 3% in Meckel's diverticulum (1–5).

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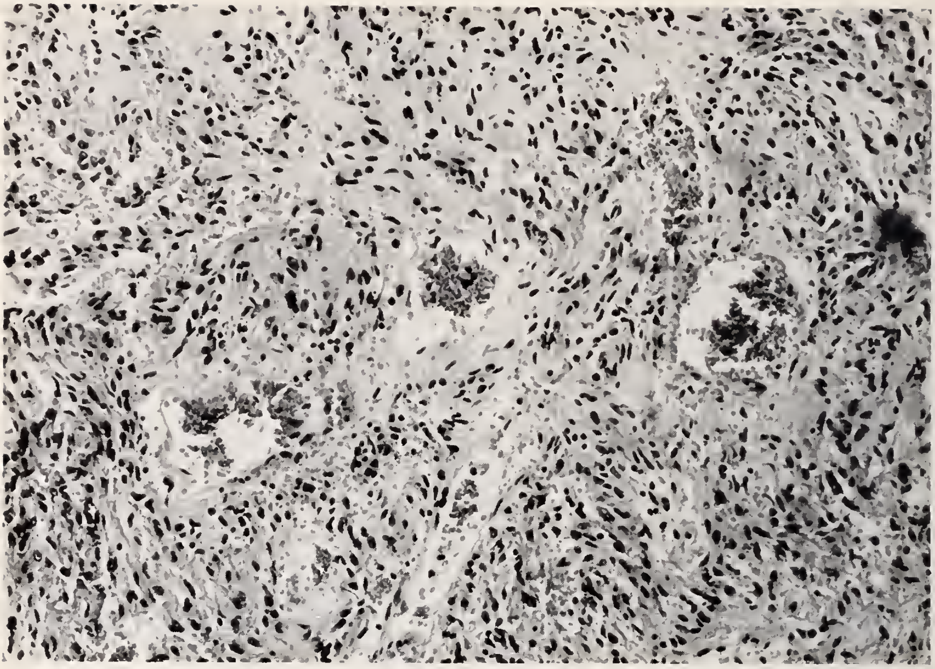

FIG. 1. Spindle-shaped smooth muscle cells are seen interlaced with connective tissue. Areas of hemorrhage between the tumor cells are seen.

No sex differentiation, familial tendencies or racial differences are noted. Diet has no known influence. The tumors are not estrogen-dependent, as are fibromyomas of the uterus (4). The majority of patients are in the 40–60 age group (3–6).

Both the benign and the malignant tumors tend to be large, bulky tumors, yellow-white in color and firm and rubbery in consistency. Histologically, whorls of spindle-shaped smooth muscle cells are seen interlaced with varying amounts of connective tissue. The sarcomas tend to be heterogeneous and vary greatly as to the degree of anaplasia. Hypercellularity, palisading of nuclei, hyperchromasia, pleomorphism of nuclei, and the presence of increased numbers of mitotic figures are noted (7). Differentiation on frozen section between benign leiomyoma and malignant leiomyosarcoma is particularly difficult (7, 8).

About one-half of the reported cases are primarily exocentric; about 20% are endocentric. Fifty percent of the surgical specimens are greater than 10 cm in diameter. The larger tumors outgrow the blood supply and develop foci of central necrosis. These foci may coalesce, resulting in cavitation of the tumor. When the cystic tumor ruptures into the intestinal lumen, gastrointestinal bleeding occurs. Rupture into the peritoneal cavity occurs but it is rare.

Hemorrhage and pain are the most common presenting symptoms. Significant bleeding is noted in 65% of patients with duodenal leiomyomas; 73% of patients with jejunal leiomyomas; 35% of patients with ileal leiomyomas; and 50% of patients with Meckel's leiomyomas. The hemorrhage may be fatal and often shows little tendency to stop (5, 6). Pain is a prominent feature in approximately 50% of patients with small intestinal leiomyomas (5). Pain results when the tumor causes partial intestinal obstruction, torsion of the bowel, kinking or intussusception (8). Other presenting symptoms include nausea, vomiting, anorexia, constipation, hematemesis, indigestion, gas bloating and fever of unknown origin. Fistula formation and perforation have been reported but are extremely rare. Palpation by the examining physician has been the cause of perforation. Perforation with spillage of blood and intestinal contents into the peritoneal cavity is a life-threatening emergency (5, 6, 8, 10, 11).

Accurate diagnosis is difficult. Fewer than 30% of intestinal leiomyomas are palpable (5). Contrast studies establish the diagnosis in 89% of patients with tumors of the duodenum; 44% of tumors of the jejunum; 54% of tumors of the ileum; and 25% of Meckel's diverticulum.

Both the benign leiomyomas and the malignant leiomyosarcomas present with similar clinical symptoms. At surgery, unless obvious metastases are present, differentiation may often be difficult (7, 8). Wide excision of the tumor is therefore recommended (8, 9).

This case shows that benign leiomyoma of the ileum may cause intermittent lower intestinal bleeding for several years before the diagnosis is established. The diagnosis will be missed if the lesion is not detected radiographically.

If small intestinal leiomyomas are considered more actively in the diagnosis of abdominal pain and lower gastrointestinal bleeding, the diagnosis will be made with increasing frequency, thus avoiding the consequences of massive bleeding and perforation.

Summary

A case of cystic leiomyoma of the ileum presenting with recurrent episodes of bleeding and eventually free perforation and generalized peritonitis in an 86-year-old woman is reported. The characteristics of leiomyomas of the intestine and the pertinent literature are reviewed.

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Radionuclide Imaging:

An Unusual Cervical Lesion—Parathyroid Cyst

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Encountering an unusual case of a lateral cystic neck mass in a fifty-six-year-old woman led us to review the literature for previous experiences with this definite entity of radioisotopic and surgical significance.

Parathyroid cysts are rare; approximately sixty cases have been reported.

Although the literature frequently cites Goris (1) as having first described this lesion in 1905, actually Sandstrom described it in 1880 (2).

Since the diagnosis is rarely made preoperatively or at the time of surgery, this case is being submitted in order to bring this entity to the attention of the various physicians who may encounter this lesion.

Case Report

A 56-year-old-woman was admitted with the history of having an enlargement in the region of the left lobe of the thyroid gland for eleven years. Recently she sought advice because of further increase in size and a "sticking" sensation in the throat.

Physical examination revealed a 5′ 3″, 130 lb. middle-aged woman in no distress, with a blood pressure of 140/85, pulse 80, and a respiratory rate of 18. Within the neck, in the region of the left thyroid lobe, was a cystic, firm, 8-cm mass. There was no bruit, but the pulse was transmitted through the mass. The vocal cords were normal by indirect laryngoscopy.

The complete blood count, sedimentation rate, urine, and chemical profile which included the serum calcium, phosphorus, and alkaline phosphatase were within normal limits.

An I_{131} thyroid scan (Fig. 1) showed a large, cold nodule in the region of the left inferior thyroid pole. It caused ironing-out of the remainder of the left lobe with displacement of the entire thyroid to the right. Differentiation could not be made between an intrinsic and extrinsic lesion.

At surgery, a cystic mass was found at the lower left lobe of the thyroid beneath the strap muscles. It was thin-walled, filled with clear fluid, and displaced the thyroid superiorly and lay on the recurrent laryngeal nerve (Fig. 2). The cyst was easily removed and was entirely outside the thyroid capsule.

The patient's postoperative course was satisfactory and she was discharged on the 3rd postoperative day. Her follow-up examinations were unremarkable.

Microscopic examination of the tissue displayed a thin cyst wall composed of fibrous connective tissue in which scattered clusters of parathyroid cells were present. The inner lining was composed of cuboidal epithelial cells (Fig. 2).

Discussion

The majority of patients with parathyroid cysts have asymptomatic neck masses. A few cases have been reported in which patients complained of

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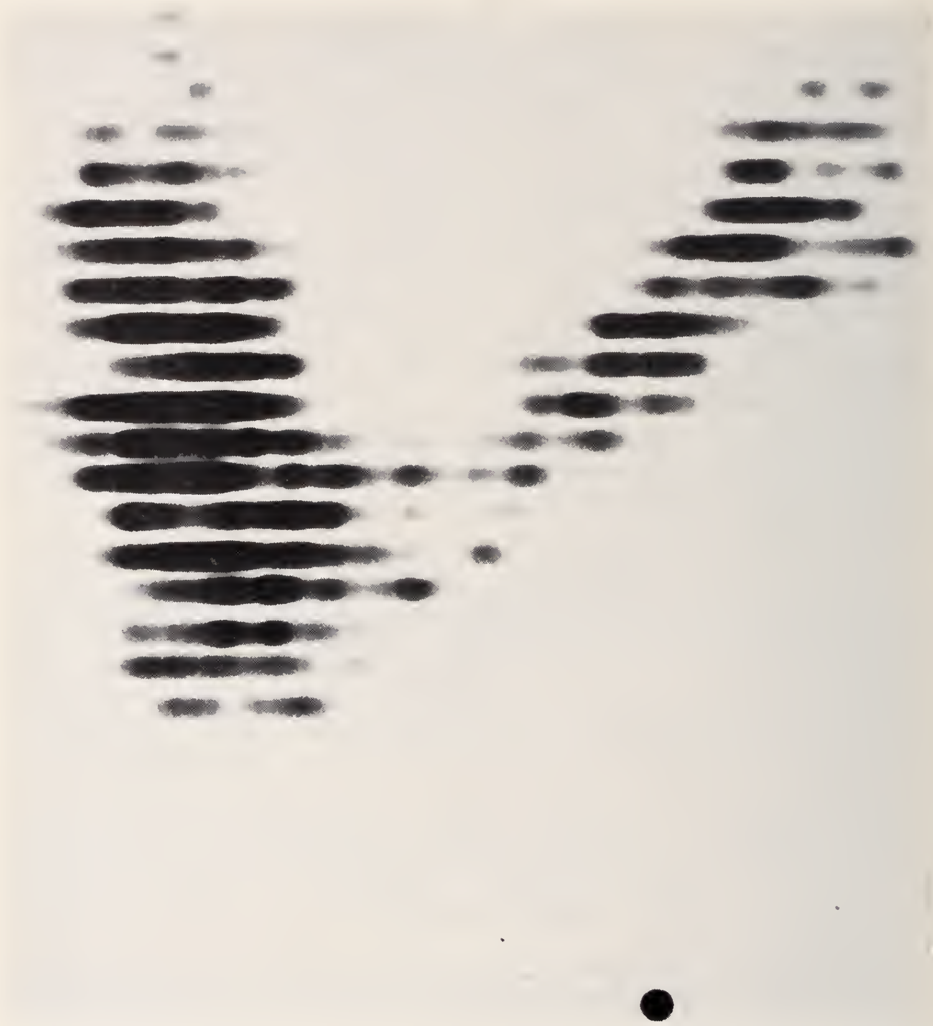

FIG. 1. I_{131} scan showing a large cold nodule involving the inferior pole of the left thyroid lobe.

dysphagia, dyspnea, or were found to have paresis of the recurrent laryngeal nerve or tracheal compression.

Most cases display no evidence of hyperparathyroidism. In those cases that have, the cyst has been associated with cystic degeneration in a parathyroid adenoma (3–6).

Parathyroid cysts generally occur in relation to the inferior pole of the left thyroid. They predominate in women, especially in the fourth, fifth, and sixth decades.

I_{131} scintiscans have revealed a cold area corresponding to the cystic lesion. Ordinarily, these cannot be differentiated from intrinsic thyroid cold nodules.

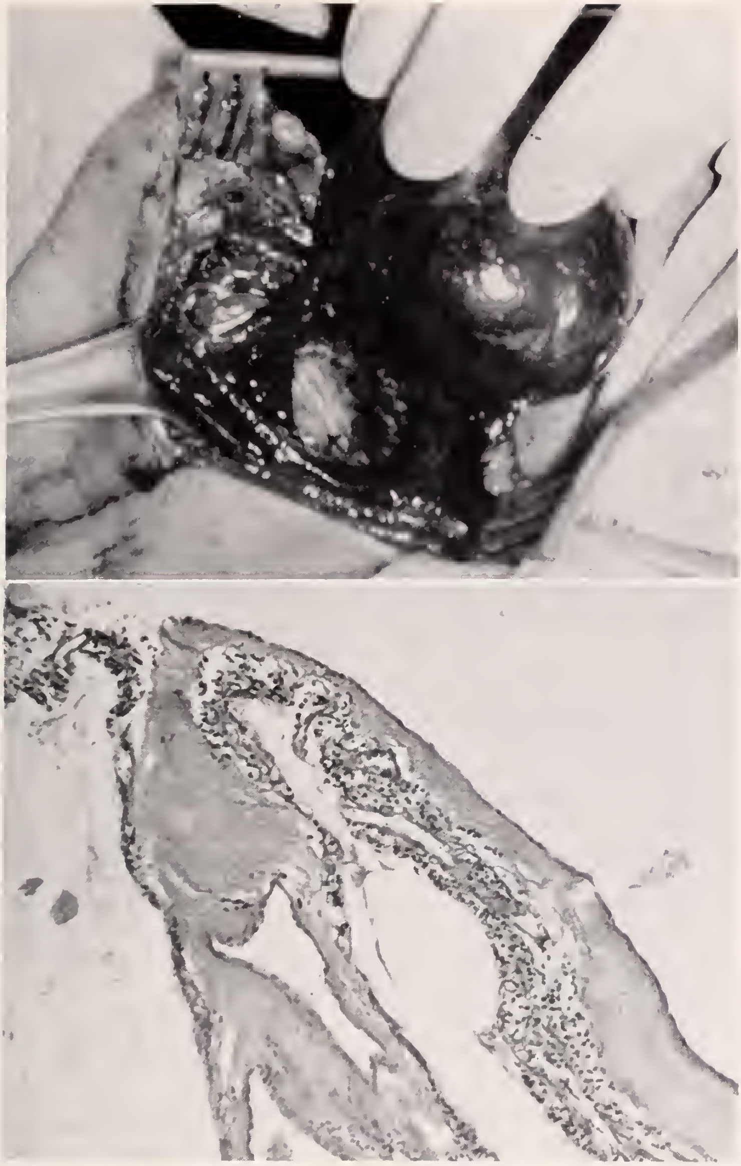

FIG. 2. Upper right: A large fluctuant smooth cyst found at lower left lobe of thyroid; Lower left: Cyst wall lined by cuboidal epithelium with a fibrous tissue wall containing clusters of parathyroid cells.

Differentiation would be helped by multiplicity of the cold nodules or by uptake in functioning thyroid tissue surrounding the cold nodule. Since these parathyroid cysts cannot be differentiated from other thyroid lesions by scintigrams, one should include in the differential diagnosis the possibility of a parathyroid cyst, especially when the lesion is fluctuant.

There are several predominant theories of origin of these cysts (7, 8). Embryologic pharyngeal pouch remnants, Kursteiner's canals, may be incorporated within the parathyroid glands and subsequently give rise to these cystic lesions. Coalescence of microcysts within parathyroid tissue and cystic degeneration in a parathyroid adenoma are other possible origins. The latter may account for the rare case of hormonally functioning parathyroid cysts.

At surgery, these cysts are usually semi-transparent, thin-walled, and loosely attached to the thyroid. The majority contain non-sticky, clear fluid and are anterior to the recurrent laryngeal nerve.

The microscopic findings include a simple cyst generally lined by cuboidal epithelium. The wall is composed of fibrous tissue and displays aggregates of parathyroid cells which may be few in number. It is important that the pathologist examine the wall of the cyst closely when confronted with the above clinical and radioisotopic findings.

Summary

The recognition of parathyroid cysts may be simplified by the awareness of clinical and morphologic data. Although of rare occurrence, they should be considered in the differential diagnosis of single cold nodules on a thyroid scan.

A case history is presented and the clinicopathologic findings are described.

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Lactic Acidosis in Acute Leukemia

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Lactic acidosis has been recognized in patients with a variety of diseases (1, 2). In some patients there may be an obvious underlying metabolic disorder, usually related to hypoxia. In other patients there is no known cause (3). We observed a child with acute leukemia who developed severe lactic acidosis terminally.

Case Report

A 12-year-old Black girl was diagnosed as having acute lymphoblastic leukemia in April, 1972. She had anemia and a leukocyte count of 800,000/mm^3 with 95% lymphoblasts. She received prednisone 60 mg/day and vincristine 3 mg/week. Five doses of intrathecal methotrexate were also given. Since she showed only partial remission on bone marrow examination after 6 weeks of therapy, 6-mercaptopurine and methotrexate were added to the regimen. In June, many blasts were still present in smears of blood and bone marrow and moderate hepatosplenomegaly was noted. She was given a course of *l*-asparaginase, prednisone, and vincristine. Bone marrow remission was attained. She was then maintained with cyclophosphamide. Late in August bleeding gums, fever and marked hepatosplenomegaly were noted and bone marrow showed leukemic relapse. Platelet transfusion and cephalothin were given. A few days later increasing fever and rapid breathing were observed. Respirations were 40/min. and there was obvious air hunger. Chest x-ray and neurologic examination were negative. Leukocyte count was 98.200/mm^3, with 95% blasts, hemoglobin was 7.1 gm/100 ml; and platelet count was 20,000/mm^3. Hemoglobin electrophoresis showed only hemoglobin A.

The patient was placed in an oxygen tent and intravenous oxacillin and gentamicin were begun, but fever persisted. Necrotic ulcers appeared on the vulva from which *Escherichia coli* was cultured. The same organism was isolated from the blood culture. The vulvar ulceration progressed and the patient's condition deteriorated despite treatment. She received three injections of daunorubicin. Blood gas determinations were done on September 16 because of severe tachypnea. Blood pH was 7.1, pO_2 116 mm Hg, and pCO_2 was 7 mm Hg. This marked metabolic acidosis did not respond to large intravenous doses of sodium bicarbonate (2 mEq/Kg every 12 hours). A blood specimen obtained on September 19, the day before death, showed marked increase of serum lactic acid (91 mg%; normal 3–12), severe decrease of p50 O_2[†] (19.6 mm Hg; normal 26–27), with a blood pH of 6.9. The red cell 2,3-DPG was markedly decreased (1.01 μmoles/ml RBC μmoles/ml RBC; normal 4.2 ± 0.2), but the ATP was elevated (2.78 μmoles/ml RBC; normal 1.4 ± 0.2). Other abnormal blood chemical determinations observed during the last day of life included: BUN 64 mg%, sodium 153 mEq/l, potassium 2.9 mEq/l, CO_2 3.0 mEq/l, chlorides 123

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[*] Supported by grant AM 14697-03 from the National Institutes of Health, Bethesda, Maryland, and the Children's Blood Foundation, Inc.

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[†] p50 O_2 = oxygen tension when hemoglobin is half-saturated.

mEq/l, calcium 7.5 mg%, phosphorus 8.6 mg%, total protein 5.2 gm%, albumin 2.49 gm%, creatinine 2.6 mg%, SGOT 380 sigma units, pO_2 118 mm Hg, pCO_2 8 mm Hg, and pyruvate 220.5 mg% (normal 300–900). Postmortem blood culture showed heavy growth of *Pseudomonas aeruginosa*. The final clinical diagnoses were: acute lymphoblastic leukemia, lactic acidosis (terminal), renal failure, and pseudomonas sepsis. Autopsy revealed diffuse interstitial leukemia infiltrates in the lungs, liver, spleen, and kidneys, and ulceronecrotic vulvovaginitis.

Comment

In several patients with acute leukemia in relapse elevation of the blood lactate levels has been reported. Lactic acidosis has in some instances developed in spite of apparently adequate oxygen supply to the tissues (4, 5). Coincident with a favorable response to antileukemia chemotherapy, lactic acidosis diminished in these patients. In another study it was found that the serum lactate level of children with leukemia is higher during leukemic relapse than during remission (6). Calculations based on in vitro anaerobic acid production by acute leukemic leukocytes suggest that large aggregates of leukemic cells, crowded into organs and metabolizing anaerobically, may produce sufficient lactic acid to increase the blood lactate concentration markedly and to cause acidosis.

Profound acidosis, renal failure, and pseudomonas septicemia occurred as a terminal event in this patient and was associated with severe depression of red cell 2,3-DPG (7). When the p50 O_2 was corrected to standard conditions in vitro (pH 7.4), a marked increase in the oxygen affinity of blood was noted. A linear relationship between values for 2,3-DPG and p50 O_2 has been determined in a number of clinical states associated with hematologic and endocrine disorders (8). A change of 430 $m\mu$moles of 2,3-DPG per ml of red cells results in a concomitant change of 1 mm in the p50 O_2 of the whole blood (9). The findings in our patient confirm this relationship. Decreased 2,3-DPG and increased oxygen affinity caused decreased venous oxygen tension and increased tissue hypoxia, which further compounds the metabolic aberrations in the acidotic patient. The profound acidosis per se, however, causes a decrease in oxygen affinity in vivo by the Bohr effect.

Of interest in our patient is the two-fold elevation of ATP and the hyperphosphatemia, an event associated with increased levels of ATP in the red cell. Although ATP may also decrease the oxygen affinity of hemoglobin in vitro (increase p50 O_2) the overriding effect of the acidosis and sepsis, which itself may be associated with hypophosphatemia (10), was to lower the level of DPG.

Rapid corrections in body pH cause immediate changes in oxygen affinity by the Bohr effect (11). Raising pH from 6.9 to 7.4 by infusion of bicarbonate results in an increase in oxygen affinity in vivo and decreased oxygen unloading. Tissue hypoxia may then result in further lactic acidosis. A gradual correction in pH allows time for glycolytic alteration of DPG levels and normalization of oxygen affinity. Because of the risk of circulatory overload and of the effects of sudden increase in pH on blood oxygen affinity, cautious infusion of alkali at a rate sufficient to limit but not necessarily correct the acidosis is recommended.

Summary

A child with acute lymphoblastic leukemia was presented who had severe lactic acidosis associated with increased oxygen affinity and decreased levels of red cell 2,3-DPG during the terminal phase of her disease. Clinically she showed tachypnea, renal failure, and sepsis due to *Pseudomonas aeruginosa*. Autopsy revealed severe leukemic infiltration of the liver, spleen, and kidneys. Large aggregates of leukemic cells, crowded into organs and metabolizing anaerobically, may produce sufficient lactic acid to cause acidosis. Decreased 2,3-DPG and increased oxygen affinity cause decreased venous oxygen tension and increased tissue hypoxia, thus producing more acidosis.

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Segmental Phlegmonous Colitis: A Rare Complication of Nephrointestinal Fistula

Report of a Case and Review of the Literature

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Transmural segmental phlegmonous colitis is an entity characterized by an acute edematous inflammation of all coats of the colon. It is usually related to a subacute perforation by a foreign body which becomes lodged at a flexure.

In addition to the obvious passage of objects (fishbone, toothpick) through the colonic lumen, the less obvious passage of a gallstone or a renal calculus in the colon may present as a right upper quadrant mass with all the characteristics of the originating organ. The following case report illustrates the complications which may ensue.

Case Report

R.S. (MSH #281647), a 56-year-old woman was admitted to The Mount Sinai Hospital on February 3, 1967 with a right upper quadrant mass, fever (T = 101.4°F) and pain. The patient was known to have had a right pyelonephritis for 33 years, characterized by episodes of pyuria, hematuria, dysuria, fever and flank pain. Intravenous urogram six years prior to admission demonstrated a normal left kidney and a poorly visualized right kidney. In 1939, the patient underwent excision of an echinococcus cyst of the left lobe of the liver. In 1965, she was reexplored for lysis of small bowel adhesions.

On physical examination at admission, the patient had a partially movable mass located under her right upper quadrant scar which was erythematous, edematous and tender.

On an abdominal film, mottled interstitial air could be seen within this mass and it was felt that emphysema of the gall bladder, an anaerobic infection, could not be ruled out. Intravenous pyelography (Fig. 1A) demonstrated multiple calcific densities in the right upper quadrant, no renal visualization on the right and a nephrogram on the left. The patient was azotemic at the time. Emergency cystoscopy showed a trabeculated edematous bladder and the right ureteral orifice could not be identified. Pertinent laboratory studies on admission showed a BUN of 137 mg% and a creatinine of 8.6 mg%. There was marked leukocytosis (WBC 29,000) and the urine showed heavy pyuria. Urine culture grew E. coli, B. proteus, and A. aerogenes, sensitive to various antibiotics. Blood cultures were negative throughout the entire hospital stay.

The patient continued to have persistent right upper quadrant pain, fever, and leukocytosis. Exploratory laparotomy the next day revealed a phlegmon of the colon adjacent to a right perinephric abscess communicating with a calculi-containing kidney. The gall bladder was normal. The perinephric abscess was widely drained, a few calculi removed, a right nephrostomy was performed, and the right colon exteriorized.

The postoperative course was complicated by azotemia secondary to acute tubular necrosis, requiring several dialyses. The acute tubular necrosis was due to sepsis, hypotension, large bowel ischemia, and necrosis. The persistent phlegmon of the colon was finally controlled by segmental colectomy of the exteriorized segment.

By March 7, 1967 after the azotemia had subsided and the BUN and creatinine had returned to normal, an intravenous pyelogram demonstrated a normal bladder and left kidney (Fig. 1B).

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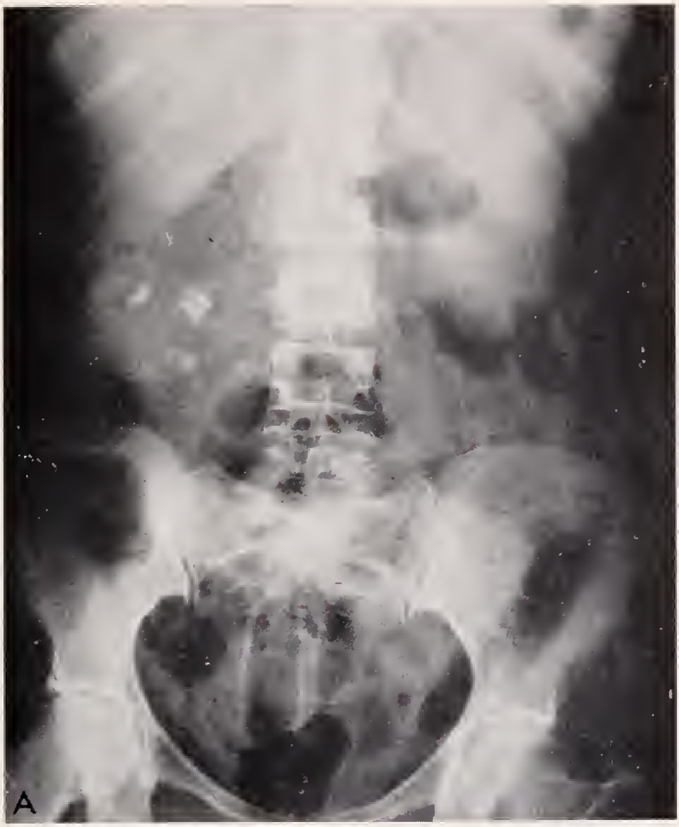

FIG. 1A. IVP on admission showing multiple calcific densities in the right upper quadrant. No renal visualization on the right and a nephrogram on the left.

The right nephrostomy tube was dislodged and purulent drainage persisted from the right flank.

On May 5, 1967 a right nephrectomy by morcellation was performed. On June 22, 1967, the exteriorized colostomies were reapproximated, the hernia repaired, and the patient discharged. She has remained well for six years after discharge.

The pathologic study of the right kidney demonstrated extensive and severe old and recent calculous pyelonephritis with marked destruction of renal parenchyma. Remnants of renal tubules without glomeruli were seen. The mucosa of the calyces and renal pelvis showed an unusual metaplastic change inasmuch as the transitional epithelium was partially replaced by mucous glands, as seen in the colonic mucosa (Figs. 2A and B). No transitional epithelium was seen. The pathologic section of colon was reported as segment of colon with extensive phlegmonous inflammation of submucosa with areas of congestion and hemorrhage. Acute inflammatory cell infiltration of serosa and subserosa were noted, but no foreign body or fishbone was found.

Etiology

Fistula formation between kidney and the intestinal tract have been reported under conditions of 1) foreign body perforation; 2) perinephric abscess; 3)

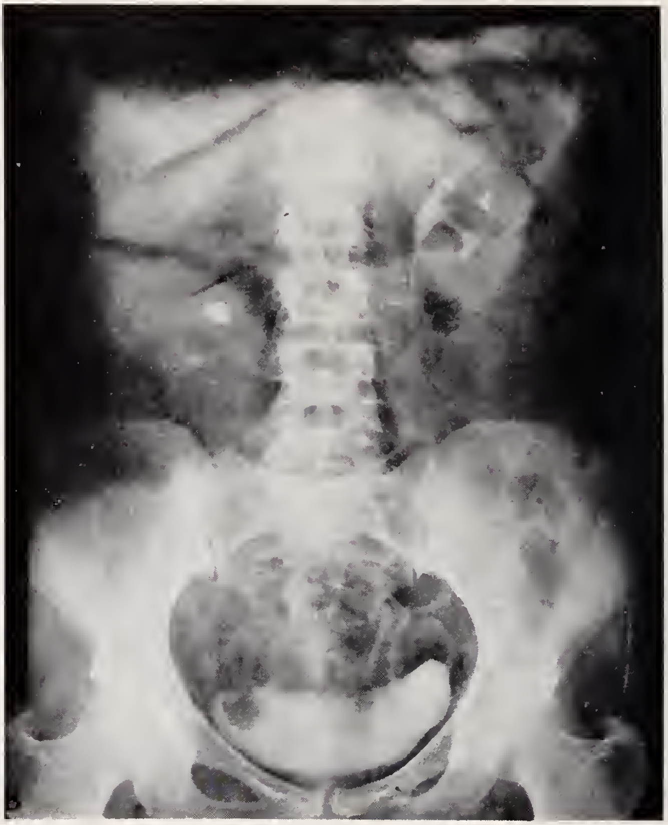

FIG. 1B. IVP demonstrating normal left kidney and bladder.

granulomatous disease of the bowel or kidney; 4) trauma; 5) mycotic infection; 6) echinococcus; and 7) surgeon injury.

Pathogenesis

A chronic inflammatory process of the upper urinary tract may lead to calculous pyenephritis and to perinephric abscess. The pus may drain along the psoas antero-inferiorly, to the skin or upward into the subdiaphragmatic space and occasionally through the diaphragm. Wharton (1) (1953) reported a case of perinephric abscess with perinephrobronchial fistula. Adhesions will form to adjacent viscera. If the abscess is antero-superior, it will track into the colon or duodenum. The progression is usually slow and involves pericolonic abscess, fixation, and then perforation. It is very rare for the abscess to dissect into the muscular coats of the bowel without perforating into the lumen. Usually the abscess perforates into the larger viscus and the tract narrows by fibrosis, leaving a small communication. The process persists, however, unless the primary nephropathy can be corrected.

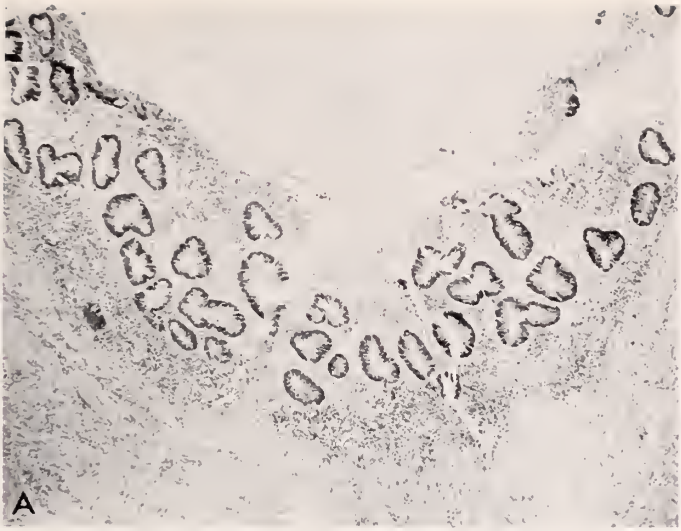

FIG. 2A. Low-power photomicrograph (× 65) showing replacement of renal pelvis transitional epithelium by mucosa lined with mucous glands.

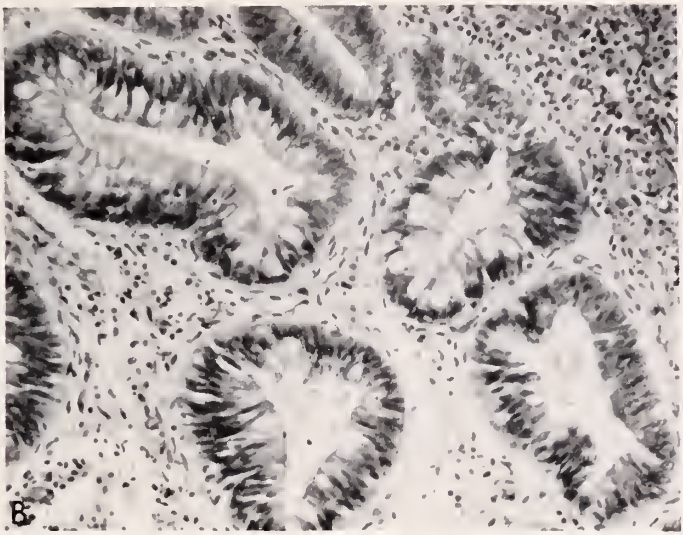

FIG. 2B. High power photomicrograph (× 200) of mucous glands replacing transitional epithelium of renal pelvis.

Surgical Therapy

This involves eradication of the source and may require nephrectomy with or without bowel diversion. Because of the chronicity and severity of the renal involvement, initial surgery may consist of drainage of the abscess and nephrostomy. Final surgery may require a process of morcellation rather than simple nephrectomy as the perinephric planes may be obliterated. Thorough drainage is a necessary concomitant of such procedure.

The intravenous pyelogram will almost always fail to show visualization on the affected side. Retrograde urography may demonstrate the abscess cavity and fistulous tract.

Clinical Picture

Since the 1940's with the advent of tuberculous chemotherapy, the majority of abscesses are related to nontuberculous, calculous pyonephrosis or pyelonephritis. The patient is often chronically ill with recurrent chills, flank pain, tenderness. Electrolyte imbalance, gastrointestinal problems, and renal failure may complicate the picture.

Discussion of the Literature

Renocolonic fistula was first mentioned as an entity in the writings of Hippocrates (2) in 450 B.C. as a passage of kidney stones into the bowels. An extensive review of the literature of renocolonic fistula was made by Mertz (3) going back as far as 1767. Vermooten and KcKeown (4), Wesson (5), Ratliff and Barnes (6), Findlay (7), Ellick and Getz (8), Narins (9), Goodwin (10), Ehrenfeld (11) and others (12, 13, 14) added new cases to the literature.

The etiologic factors leading to nephrocolonic fistula are eroding calculous disease, perinephritis, and tuberculosis. Chronic renal inflammation produces a suppurative renal process, perinephritis or perinephric abscess. The close anatomic relationship between kidney and colon explains why nephrocolonic fistula is the most common renovisceral fistula. About one-third of the anterior surface of the kidney is in contact with the colon. The majority of the cases exhibit fistula formation from the cortex of a calyx rather than from the renal pelvis. The inflammatory process is primarily located in the kidney and fistulization from the bowel into the kidney is practically unknown.

The patients presented in the literature usually have a recurrent, longstanding urinary tract infection. At one point or another a perinephric abscess forms, may seemingly resolve and instead progress to erosion and fistulization into the bowel. The signs and symptoms of fistulization consist of the appearance of blood, pus, urine, and calculi in the stools.

Malignant renal tumors may erode the bowel and involve either the ascending or descending colon, but they rarely cause fistula (Goodwin) (10). The development of a fistula between the upper urinary tract and the bowel is usually related to urinary obstruction and infection with perforation of a hydronephrosis, development of a perinephric abscess and finally erosion of the abscess into the bowel with subsequent drainage and fistula formation (Narins) (9).

Summary

A case of segmental phlegmonous colitis secondary to calculous pyelonephritis and perinephric abscess, is presented. The most striking finding was the

complete replacement of the renal pelvis transitional epithelium by colonic-type mucosa lined with mucous glands. This was a most unusual metaplastic change, showing the wide-range potential of transitional epithelium. Renocolonic fistula today is rarely due to tuberculosis, but mainly to calculous disease and perinephric abscess formation. Although rare, renocolonic fistula is the most common renovisceral fistula.

A staged surgical approach is usually necessary.

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"Ascites" and Inguinal Hernias: Unusual Presentation for Chronic Granulomatous Disease of Childhood*

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Chronic granulomatous disease (CGD) of childhood, a genetic disorder of neutrophil (1–3) and monocyte (3) bactericidal competence, tends to manifest itself early in life with severe chronic and recurrent infections (2–7). These infections most commonly are due to Staphylococcus aureus. However, organisms of ordinarily low-grade virulence frequently infect these patients (4). Abscesses and granuloma formation occur, particularly in lymph nodes, lungs, bone, liver, spleen and skin. Transient pericarditis is relatively frequent in some series of patients (2), as is peritonitis (5). It is therefore understandable that an apparently noninfectious disorder would not raise the suspicion of underlying CGD. For this reason we are reporting a child with CGD who had what initially appeared to be ascites and bilateral inguinal hernias.

Methods

The nitroblue tetrazolium test was performed according to the method of Baehner and Nathan (1). Glucose oxidation studies were done as described previously (8). Bactericidal assays were carried out using Staphylococcus aureus and Escherichia coli as test organisms. The procedure has been modified from Quie et al (9) and has been reported (7).

Serum immunoglobulin G, A, and M concentrations were determined by the radial immunodiffusion technique, using Hyland immunoplates (Hyland Laboratories, Costa Mesa, California). Normal values for age in our laboratory are essentially the same as those reported by Stiehm et al (10).

Case Report

A 19-month-old white male was hospitalized because of progressive painless abdominal enlargement for eight days and bilateral inguinal hernias. At six months of age mild anemia was detected that improved only slightly with adequate doses of oral ferrous sulfate. At 18 months of age, a right inguinal hernia was noted. Surgical correction was deferred because of anemia.

On admission, the child appeared slim and pale but in no distress. Temperature was

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100.4°F, pulse 118/min, respirations 36/min, blood pressure 120/70, and weight 23 lbs. Physical examination revealed a 2 x 3 cm patent anterior fontanelle and generalized shotty nontender lymphadenopathy. The abdomen was soft and distended, with a questionable fluid wave. The liver was 3 cm below the right costal margin and the spleen tip was just palpable. There were bilateral, easily reducible inguino-scrotal hernias.

The hemoglobin concentration was 8.8 gm/100 ml, hematocrit 29%, white blood cell count 17,200/mm^3 with 58% neutrophils, 2% band forms, 7% eosinophils, 33% lymphocytes. There were 1.3% reticulocytes. Platelets were adequate on smear. Urine analysis was normal. Blood urea nitrogen, serum calcium, phosphorus, alkaline phosphatase, bilirubin, and glutamic oxalacetic transaminase were within normal limits. Serum total protein was 7.8 gm/100 ml with albumin 3.2 gm/100 ml. Catecholamine metabolites in a 24-hour urine specimen were normal. A normocellular bone marrow sample revealed no tumor cells.

Two days after admission, a diagnostic paracentesis was productive of brown-yellow-tinged fluid with a specific gravity of 1.033. It contained 5,200 white cells/mm^3 with 19% neutrophils, 77% lymphocytes and 4% monocytes. The protein concentration was 5.5 gm/100 ml with albumin 3.4 gm/100 ml and globulin 2.1 gm/100 ml. A cell block was negative for tumor cells and no organisms were seen on a gram-stained smear. Culture of the peritoneal fluid grew an anaerobic Streptococcus and Serratia marcescens which were initially considered to be contaminants.

On the day of the paracentesis, an intravenous pyelogram was done via a cut-down. Shortly thereafter the patient's fever spiked to 104.8°F which was ascribed to contamination at the cut-down site. He received intravenous ampicillin 200 mg/Kg/day and his fever subsided within 36 hours.

The intravenous pyelogram was normal. A chest roentgenogram showed elevated diaphragms; abdominal films showed separation of small intestinal loops; a gastrointestinal series showed an abnormal small bowel pattern with flocculation and segmentation of barium suggestive of a malabsorption syndrome. Bone age appeared retarded at age six months; there was no evidence of skeletal metastases.

On the eighth hospital day, consultation was sought since exploratory laparotomy was being considered to exclude occult neoplasm as a cause of the persisting "ascites." At this time residual minimal facial pigmentation was noted. This was related to recurrent furunculosis for 8 months which had been treated with topical antibiotics. The "ascites" now was definitely improving. The Enterococcus and Serratia in the peritoneal fluid culture which had been regarded as contaminants were now considered the cause of a low grade peritonitis that was being treated effectively by ampicillin.

Serum immunoglobulins determined at this time were elevated for age (IgG 1300 mg/100 ml, IgA 200 mg/100 ml and IgM 300 mg/100 ml).

Neutrophil function studies (Table I) on cells from the propositus indicated marked impairment in glucose oxidation, nitroblue tetrazolium reduction and bactericidal capacity for the two organisms tested. Neutrophils from the patient's mother showed some impairment in bactericidal capacity for E. coli; difficulty in demonstrating heterozygotes for CGD has been noted in previous studies (2–7). Cells from the patient's father and sister were normal in the tests of neutrophil function performed.

Subsequent Course: The low grade peritonitis subsided completely. He was discharged after a two-week course of ampicillin. His hernias persisted. For the next 37 months, the boy's clinical course has been consistent with that of a patient with CGD. His chest roentgenogram now has moderate persistent hilar lymphodenopathy. He has had several episodes of pneumonia as well as suppurative cervical and inguinal adenitis with concomitant anemia and leukocytosis. One episode of cervical adenitis required adenectomy. His liver and spleen are moderately enlarged. Prolonged antibiotic therapy, usually with chloramphenicol after other antibiotics have failed, has been associated with clinical as well as hematologic improvement. Height and weight have both improved to over the 10th percentile.

TABLE I

Neutrophil Function Tests

	Healthy Controls (✱ 30)	Propositus	Mother	Father	Sister
^{14}C-Glucose Oxidation[1] (whole blood)	11.4 ± 0.8 S.E.M.	1.2	13.1	16.3	9.2
Nitroblue Tetrazolium Test (Δ O.D.)[2]	0.21 (range 0.16–0.58)	0.03	0.18	0.21	0.35
Bactericidal capacity[3]					
Staphylococcus aureus	>2.0	1.1	2.1	3.8	ND
Escherichia coli	>3.0	1.5	2.8	3.6	ND

[1] Ratio (P/R) of $^{14}CO_2$ production from glucose 1-^{14}C by phagocytizing (P) in counts/minute and resting (R) phagocytes in counts/minute in whole blood.

[2] Optical density (O.D.) at 515 mμ (2.5 × 10^6 phagocytes per 30 minutes). (Δ equals phagocytizing minus resting O.D.)

[3] Logarithm number of organisms killed per 2 hour incubation. (Logarithm initial number of viable organisms minus final number.)

ND = Not done

Comment

A 19-month-old boy presented with what appeared to be ascites and bilateral inguinal hernias. The significance of the high specific gravity and protein concentration of the peritoneal fluid indicative of an exudate initially was not appreciated. These findings as well as the peritoneal fluid culture growth of Enterococcus and Serratia at first were not felt to be related to infection because of the lack of abdominal signs of inflammation. When these findings were reappraised, especially since the child also had recurrent furuncles, anemia, leukocytosis and hypergammaglobulinemia, neutrophil dysfunction was suspected. NBT, glucose oxidation and bactericidal function tests established the diagnosis of CGD of childhood.

Several investigators have demonstrated that neutrophils from patients with CGD have impairment in glucose consumption, a decreased respiratory burst, and impaired generation of hydrogen peroxide during phagocytosis (9, 11, 12). Although these metabolic studies are of importance in establishing the diagnosis as illustrated in the case reported here, the precise enzymatic defect in this disease still remains unknown. There are conflicting studies concerning the possible role of NADH oxidase, NADPH oxidase, and glucose-6-phosphate dehydrogenase in the etiology of this disease. These studies have recently been reviewed (11, 12).

After acceptance of this manuscript, when the patient was 5 years and two months of age, he developed a pernicious, relapsing bilateral pneumonitis with empyema. Pseudomonas cepacia repeatedly was cultured from pleural fluid. In spite of intensive therapy with appropriate antibiotics and multiple transfusions of HL-A compatible white cell concentrate obtained from the patient's father and paternal aunt, the patient died. Autopsy revealed severe necrotizing bilateral pneumonia with Pseudomonas cepacia.

We wish to thank Dr. Isaac Djerassi of the Fitzgerald-Mercy Hospital in Philadelphia, Pennsylvania, for preparing the white cell concentrates for this patient.

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Automation of Immune Hemolytic and Complement Reactions by AutoAnalyzer. III*

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This is Part III of a three-part work. Part I appeared in the March–April issue. Part II appeared in the May–June issue. The references for the entire manuscript appear at the end of this Part and anyone requesting a reprint will automatically get a reprint of all three Parts.

The AutoAnalyzer is manufactured by Technicon Instruments Corp., Tarrytown, N.Y. 10591.

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PART III

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Abbreviations and Symbols

A	Amboceptor—hemolysins, antibody against *E* which interacts with *C*
Ab	Antibody
AC	Anti-complementary substance
Ag	Antigen
C	Complement
C1, C2, ···	1st, 2nd, etc. components of complement
CH50 (CH100)	Units of complement yielding 50% (100%) hemolysis
CF	Complement fixation
CFR	Complement fixation reaction
D	Serum dilution
E	Erythrocyte = red blood cell
*E**	Erythrocyte damaged by complement
EA	Sensitized erythrocyte
EAC	Immune hemolysis due to interaction of E + A + C
GPS	Guinea Pig Serum
HD	Hemolytic Dose
MHC	Minimal hemolytic concentration
MHD	Minimal hemolytic dose
OD	Optical Density
R	Receptacle
RBC	Red blood cells
SRBC	Sheep erythrocytes
t	Time or duration of a reaction
T	Temperature usually C°
VBS 2+	Veronal buffer solution with added Ca^{2+} and Mg^{2+}

Abstract

This report is a summary of research and development involving complement fixation and immune hemolytic reactions evaluated by AutoAnalyzer. It includes description of adaptations by which this equipment can be used in serologic and serodiagnostic laboratories, experiments on temperature gradients, automatic serial dilutions, titration of complement components, the kinetics of complement fixation reactions, and the problem of anticomplementary sera.

7) *ANTICOMPLEMENTARY EFFECTS*

An anticomplementary effect is an operational term. When one performs complement fixation reactions (CFR) a certain number of "controls" with antigen and with antibody-containing serum are needed. If these controls inhibit hemolysis, the antigen and/or the serum to be tested are labeled anticomplementary. Any substance which inhibits hemolysis is considered anticomplementary even if this substance does not act directly on complement. Two different types of anticomplementary effects are seen when CFR is analyzed by automatic methods (17, 78).

1. *Anticomplementary Effect of Aggregated γ Globulin*

The anticomplementary activity of aggregated γ globulin was studied by Christian (79) and by Marcus (80). Commercial preparations of human γ globulin containing 165 mg protein/ml were utilized. These preparations were heated to 63°C for 15 minutes. Anticomplementary activity was very strong. The kinetics of the anticomplementary activity of aggregated human γ globulin were studied using the method of Fig. 5-1. The receptacle, R, received 10 ml of VBS 2+ solution, sufficient guinea pig serum to obtain minimal hemolysis, and, at time zero, 0.1 ml of a dilution of heat aggregated γ globulin. A curve was obtained exactly similar to the kinetic curve of complement fixation by immune complex (Fig. 7-1).

In this kinetic experiment, 0.1 ml of aggregated γ globulin diluted 1/100 (0.165 mg aggregated γ globulin) was introduced into a final volume of 10 ml. The exponential type curve obtained was characterized by its tangent to the origin; this was 1.2, while the "constant of the machine" was 12. The time constant, a, therefore was $1.2/12 = 0.1 \text{ min}^{-1}$. To take into account concentration of aggregated γ globulin, the rate of inactivation was calculated for a concentration of 1g/l. From $kD = a$, with $D = 0.165$ mg/10 ml, $k = 6.06$ min^{-1} for a concentration of 1 g aggregated γ globulin/liter. k can be calculated per mole γ globulin per second:

$$k = \frac{6.06 \times 160{,}000}{60} = 1.6 \times 10^{4} \text{ liters mole}^{-1} \text{ sec}^{-1}$$

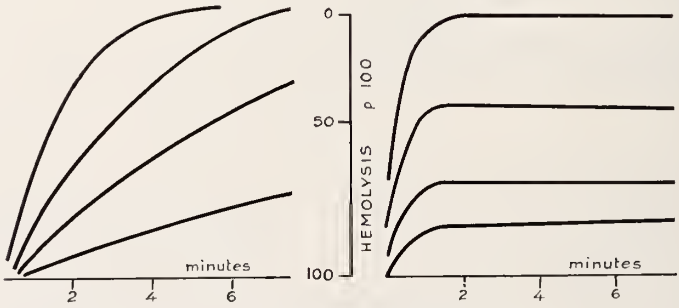

FIG. 7-1: *Two Kinetic Types of Complement Inactivation.* Left: inactivation by specific fixation. Right: inactivation by nonspecific anticomplementary effect.

This value, independent of technique, is characteristic of the anticomplementary activity of the preparation studied.

2. *Anticomplementary Effect of Aged Human Serum*

Some human sera are anticomplementary; such sera can be obtained artificially by heating at 37°C for several days. With such sera the curve obtained in a kinetic experiment is different from that obtained with aggregated γ globulin. The anticomplementary effect of old sera appears quickly and does not change with time (Fig. 7-1). In using different quantities of aged anticomplementary sera, different curves are obtained.

The anticomplementary mechanism of aged serum can be explored by sampling separately the four reactants: VBS solution, one minimal hemolytic dose of complement, hemolysin, and erythrocytes. Total hemolysis is found unless aged serum is added to one of the reactants. Furthermore, the effect of aged serum is exactly the same if it is added to any reactant. Thus, what seems to be an anticomplementary effect of aged serum is found to be an antihemolytic effect; aged serum does not appear to affect complement per se.

Table 7-1 summarizes the differences between the two anticomplementary effects. In this table it can be noted that temperature influences the anticomplementary activity of aggregated IgG, but not the anticomplementary effect of aged sera.

3. *Anticomplementary Effect of Circulating Immune Complexes*

A serum may be anticomplementary because of the presence of immune complexes. Although proposed to explain the anticomplementary effect of sera of patients with serum hepatitis, in all cases studied the anticomplementary effect gave a kinetic curve of the aged serum type while fresh serum from each patient displayed a normal complement titer.

4. *Additive Anticomplementary Effects*

A reaction described by Gajdusek (72) and named autoimmune complement fixation has been studied by traditional methods of complement fixation as well as by automated kinetic methods. The test, according to the technique of

TABLE 7-1

Anticomplementary Effect

	Aggregated γ globulin	Aged human sera
Active concentration	1/1000	1/10, 1/100
Kinetic	Effect increases exponentially with duration of incubation	Effect is stable and independent of incubation
Role of temperature	Dependent	Independent
Substances giving effect	Aggregated IgG; immune complexes	Aged sera, peptones, chemicals
Mechanism	Complement fixation	Antihemolytic activity (inhibitor)

TABLE 7-2

Test for Additional Anticomplementary Effects

	Reaction	Control Antigen	Controlled Serum
Complement (2 H100 units)	0.50 ml	0.50 ml	0.50 ml
Antigen (human liver)	0.25 ml	0.25 ml	—
Patient's serum diluted ¼	0.25 ml	—	0.25 ml
VBS 2+ Solution	—	0.25 ml	0.25 ml
	Incubate for 15 hours at 6°C and then for 15 minutes at 37°C		
Sensitized Erythrocytes	0.5 ml	0.5 ml	0.5 ml
	Incubate for 30 minutes at 37°C		

Kolmer, is given in Table 7-2. The result of this test indicated absence of hemolysis in the reaction tube, 95% hemolysis in the antigen control, and 95% hemolysis in the serum control, and the test appears to be positive. However, no conclusion could be drawn from the results because antigen was anticomplementary and consumed more than one H100 unit of complement. Serum was equally anticomplementary and consumed more than one H100 unit of complement. If each reagent consumed more than one H100 complement unit, the reaction did not have two H100 units of complement available and therefore cannot be interpreted.

By kinetic study, Gajdusek's test would be negative in the example chosen. Indeed, it was found that antigen was anticomplementary ($k = 0.04\ \text{min}^{-1}$), the cirrhotic serum tested was anticomplementary ($k = 0.05\ \text{min}^{-1}$), and the test for fixation was characterized by a time constant of $0.09\ \text{min}^{-1}$. However, if the test for fixation had furnished a value of $k > 0.09$, the presence of antibody in this serum could have been assumed.

5. *Procomplementary and Anticomplementary Effects of Inactivated Human Sera*

Human sera heated at 56°C may increase lysis of sensitized sheep erythrocytes by guinea pig serum. Such sera are stated to have a procomplementary activity. This activity can be demonstrated by the use of a dose of guinea pig complement leading to partial hemolysis (e.g., 60%). The addition of heated human serum may either diminish (anticomplementary effect) or increase (procomplementary effect) the degree of hemolysis. Procomplementary activity is assumed to arise from hemolysins in human serum. Both procomplementary and anticomplementary effects interfere in all complement fixation reactions, and an AutoAnalyzer provides the serologist with a precise tool for the analysis of such contradictory effects. Anticomplementary sera may be detected by:

1. Replacing the antigen preparation with VBS 2+ solution to determine the effect of serum on immune lysis.

2. Mixing normocomplementary human serum at 37°C for 30 minutes with the hypocomplementary serum, and then determining time required for 50%

hemolysis after adding 5 ml of sensitized erythrocytes. If the hypocomplementary serum is not anticomplementary with respect to human complement, the complementary activity of the mixture will be equal to the sum of the complementary activities of the two sera. If, on the other hand, the time of 50% hemolysis of the mixture is greater than the time obtained with normocomplementary serum alone, an anticomplementary effect can be assumed to be present in the hypocomplementary serum.

8) *AUTOMATION OF COMPLEMENT FIXATION REACTIONS*

The AutoAnalyzer is a new apparatus allowing precise serologic measurements. Nevertheless, a manual method should not be converted for use on the AutoAnalyzer unless the technique is suitable both for the apparatus and for the immunologic system to be studied.

1. *Quantity of Complement for CFR*

In CFR, it is not possible to use the exact quantity of guinea pig serum necessary for lysis of red cells. Actually, during the phase of fixation, part of diluted complement becomes spontaneously inactive, and sera to be tested may have a small anticomplementary effect. If too little complement is used, there will be too many false positive sera, and if too much complement is used, the test loses sensitivity and sera which are weakly positive appear negative.

In traditional serology, two H100 units of complement have been used. With automatic apparatus, all parameters are identical for each sample and a single full unit corresponding to approximately 1.2 minimal hemolytic doses is used.

Controls

In automated serology the same controls must be applied as in manual serology. The entire hemolytic system (erythrocytes + hemolysin + complement) is controlled by sampling cups filled with VBS 2+ solution instead of serum. Antigen (absence of significant anticomplementary effect) is controlled by titrating complement in its presence. Sera known to be negative and positive serve as controls for negativity and positivity. Finally, each serum which has given a positive test must be further tested for anticomplementary effect. It is not necessary to perform this control for the sera which have been found to be negative.

2. *Technique No. 1: Automated Continuous Flow with Use of Two Incubation Coils*

In this method, sera to be tested are placed in sampler cups where rinsing fluid automatically separates successive serum samples. Antigen and appropriately diluted complement are sampled continuously. The three reagents, serum to be tested, antigen, and complement are mixed and incubated in a long delay coil at 37°C for the complement fixation phase. This material is re-pumped before being introduced to sensitized erythrocytes, after which the mixture is incubated through a second delay coil in which the hemolytic phase takes place. Finally, hemolysis is detected by photometric cuvette and its degree is recorded (7, 13, 14, 24, 26). Fig. 8-1 indicates the flow diagram.

Complement is titrated by placing manual dilutions (2%, 1.8%, 1.6% …… 0.8%, 0.6%, 0.4%) of guinea pig serum into sampler cups. The minimal hemolytic concentration of complement to be used is deduced from the results of testing these dilutions of complement.

After determination of titer of complement the instrument is used as indicated in Fig. 8-1. The sampler tray is loaded with sera to be tested. Cups No. 1 and 2, however, contain 1% formaldehyde, which will give two easily discernible peaks. Approximately thirty minutes after starting, the first results are recorded. Positive sera are tested a second time, but without antigen, to check for anticomplementary activity.

The baseline of the record is at an approximate O.D. of 0.05, corresponding to total hemolysis, because the rinsing solution between serum samples does not

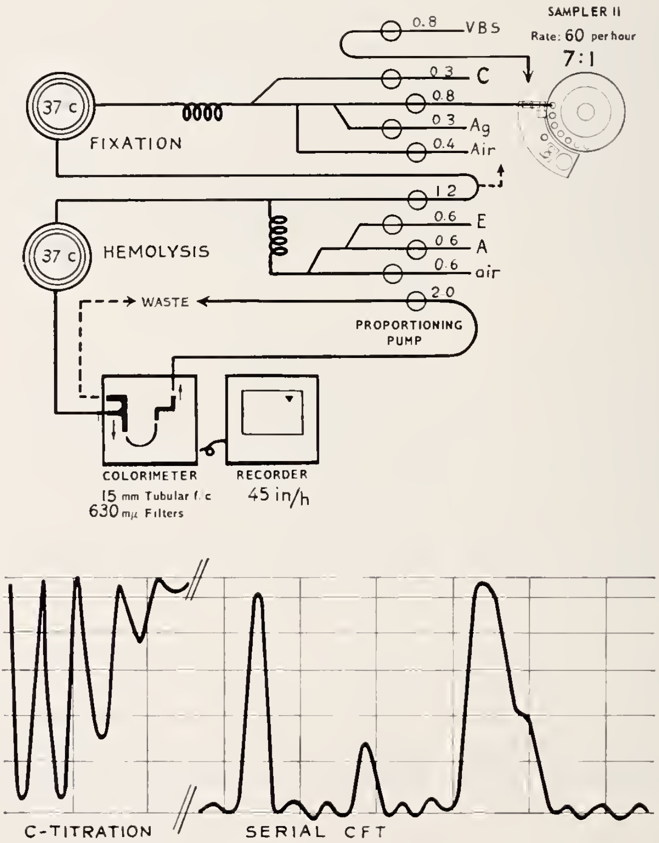
SAMPLER II
Rate: 60 per hour
7:1
0.8
VBS
0.3
C
0.8
0.3
Ag
0.4
Air
37 c
FIXATION
1.2
0.6
E
0.6
A
0.6
air
37 c
HEMOLYSIS
2.0
WASTE
PROPORTIONING PUMP
COLORIMETER
15 mm Tubular f/c
630 mμ Filters
RECORDER
45 in/h
C-TITRATION
SERIAL CFT

Fig. 8-1: *Automation of Complement Fixation Reactions with Serial Continuous Flow for Both Fixation and Lysis.* Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

affect complement. Negative sera are indicated by small deviations from the baseline while positive sera give a marked peak, the height and curvature of which are measures of the degree of positivity.

This method has inconveniences (81). The flow circuit is so long that, in the event of mistake or accident, much time is lost before normal functioning can be restored. The delay coil which serves for the fixation phase quickly becomes anticomplementary and it is necessary to clean this coil extremely carefully.

Finally, the length of circuit is responsible for contamination from a serum sample rich in antibody so that one may not be sure whether the subsequent sample is positive or negative.

This system is useful for automatic titration of antigen (71, 73). Using a flow rate of 10 to 20 per hour, each antigen is placed into a sampler cup where it is diluted automatically, continuously, and exponentially for the period of sampling. Immune serum and complement are sampled continuously and the remainder of the technique is as shown in Figure 8-1. The rate of sampling is sufficiently slow to avoid contamination between samples, and each antigenic preparation tested gives most often a sigmoid titration curve where strong concentrations lead to total inhibition of lysis. In some instances, an excess of antigen prevents fixation so that a prozone is observed.

The system is equally useful for titration of antibody which can be accomplished automatically with either a linear or an exponential continuous dilution system.

Figure 8-2 demonstrates the thermal optimal of complement fixation. Complement, antigen, and antibody each diluted for 20% lysis at 37°C were sampled continuously. At the beginning of the experiment, the delay coil for complement fixation was at 20°C, and a heating device was used so that this temperature would increase at a rate of 1°C every two minutes until 60°C was reached. An S-shaped curve was obtained. The same experiment was then

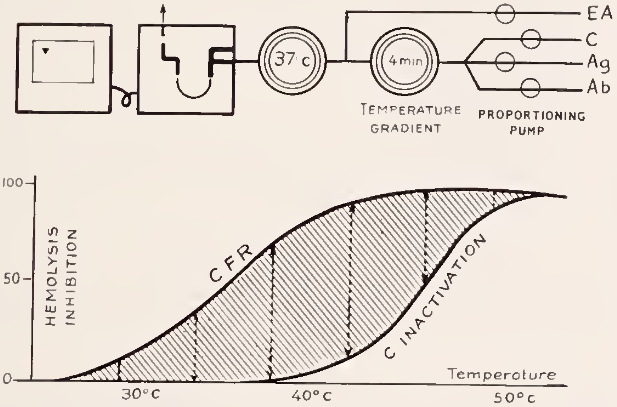

FIG. 8-2: *Automatic Determination of Optimal Temperature for Complement Fixation.* The height of the shaded zone indicates the degree of specific fixation; the maximum is located at about 42°C. Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

repeated, substituting VBS 2+ solution for antigen. This provided thermal inactivation of complement in the absence of complement fixation. A second S-shaped curve was obtained (Fig. 8-2). The difference between the two curves indicated a thermal optimum of 42°C.

3. *Technique No. 2: Semi-Automatic Method*

In this method, serum to be tested is manually diluted and introduced into sampler cups. Complement and antigen are then added automatically so that each cup receives the three reactants of the complement fixation phase. These cups are then incubated, preferably at 37°C. Finally, they are sampled to determine their residual content of hemolytically active complement. Because complement fixation has taken place under static conditions, contamination of one test by "carry over" of too much antibody from a preceding test is avoided.

The flow diagram is shown in Fig. 8-3 (12, 22, 23, 68, 81). In A, a tray contains 40 cups, each containing 0.1 ml of each serum to be tested. This tray is placed on the first sampler, B, which is furnished with a cam to provide 120 tests per hour. Here, a stationary probe distributes 0.6 ml of complement and 0.6 ml of antigen into each cup. This requires a period of 20 minutes, during which time the cups are heated to 37°C. If more time is needed at 37°C, the tray goes to C, a heated bath at 37°C. Phase C can be omitted if 20 minutes of fixation are considered sufficient. Finally, the tray is transferred to D, a second sampler, which also maintains the cups at 37°C. Here, the contents of each cup are sampled and added to sensitized erythrocytes for the hemolytic phase of the reaction.

Figure 8-4 shows the peaks yielded by this method. The baseline is at O.D. = 0.3, which represents zero hemolysis because the wash fluid sampled between serum samples does not contain complement. Thus, negative sera give deep depressions.

Advantages of this method derive from its simplicity; mistakes are easily corrected, and no reagents are wasted between samples. Duration of time for

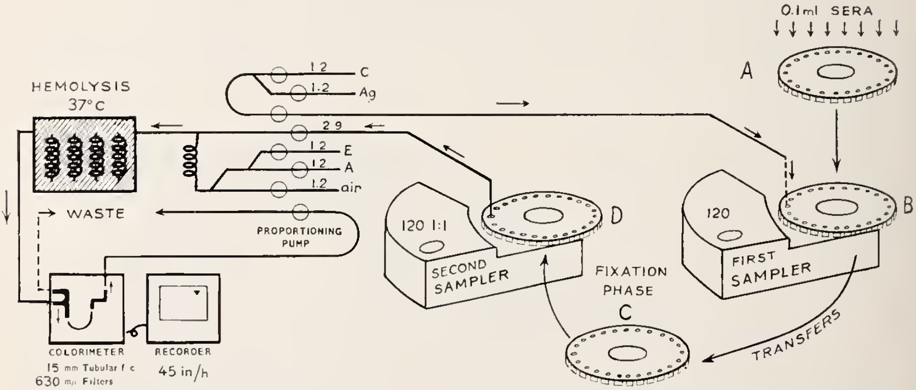

FIG. 8-3: *Automation of Complement Fixation: Transfer Method* (see text). Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

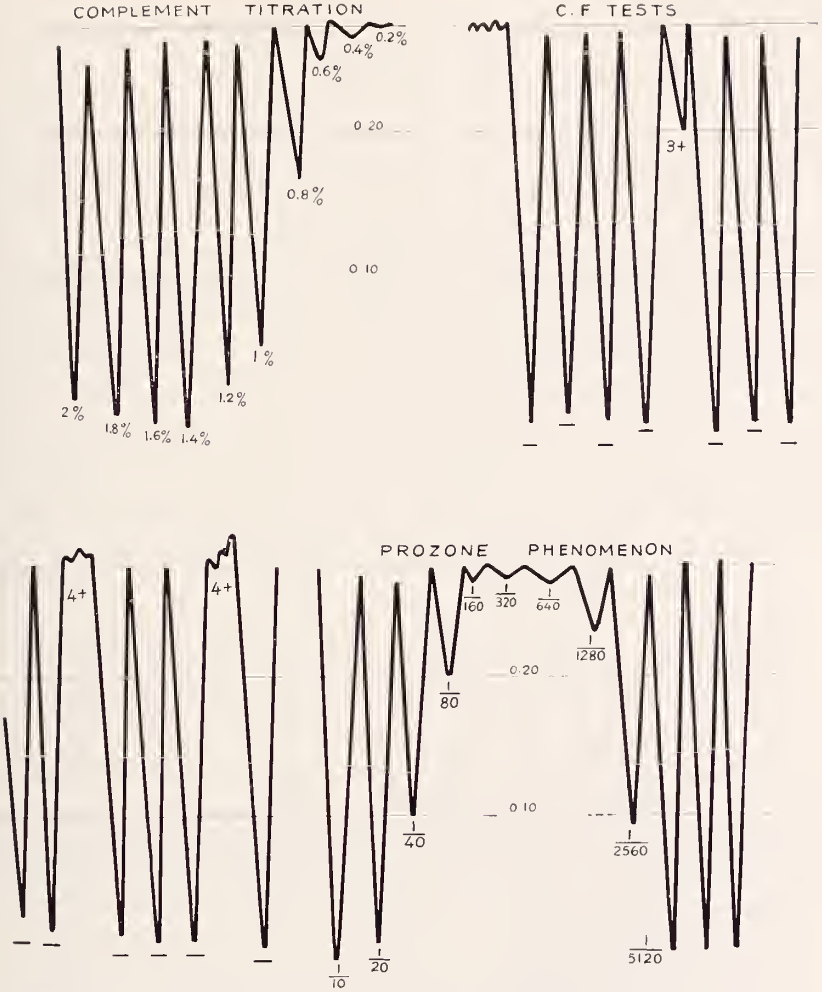

FIG. 8-4: *Graphs Obtained with Transfer Method* (see text for explanation).

complement fixation can be varied, and even temperature during this period can be regulated. Actually, even prolonged fixation in the cold is possible. Finally, there is no contamination of a negative serum by a preceding strongly positive serum.

This method is not entirely automatic, and a technician must ascertain both proper distribution of each serum and transfer of the trays. The procedure is quantitaitve for positive sera when the test is repeated at several serum dilutions.

The method also discerns prozone phenomena. For example, when it is used to detect Australia antigen, a single serum dilution for testing is unsatisfactory because sera rich in antigen show a prozone: they are negative at strong concentrations but positive when diluted. This is shown in Figure 8-4. Dilutions 1/10 and

1/20 because of antigen excess do not allow complement fixation whereas dilutions of 1/160, 1/320, and 1/640 show complete inhibition of hemolysis.

4. *Technique No. 3: Automatic Dilution and Transfer*

This method was devised to take advantage of both preceding methods and eliminate respective defects. It is entirely automatic but based on the principle of complement fixation in separate cups. It provides serial dilution automatically and thereby eliminates prozone phenomena (Fig. 8-5).

Heat inactivated sera for testing are distributed in cups on a sampler tray. If dilutions of these specimens are desired, other cups containing VBS 2+ solution are placed between serum samples, one such cup for each 10-fold dilution. These specimens and diluents are sampled at a rate of 120 tests/hour using a 1:2 ratio of sample-to-wash. Antigen and complement are sampled continuously at the same time and the mixture of the three reactants is deposited in cups positioned on a tray of a second-phased sampler also operating at 120 tests/hour but using a 1:1 ratio of sample to wash. As explained in Section 2, each specimen cup will be sampled for 10 seconds (0.05 ml of specimen) followed by sampling of the wash receptacle which contains VBS 2+ solution, for 20 seconds (0.11 ml of diluent). At the same time, antigen and complement will each be sampled continuously for 30 seconds (0.16 ml of each), so that the serum sample will be diluted 1:10. If a cup of diluent follows this serum specimen in sequence, there will be residual serum specimen in the sampling system, which has been constructed so that it will contain just enough to permit formation of a complement fixation at a dilution of 1:100. A third cup of diluent would allow for a test at a dilution of 1:1000.

On the second sampler, cups are incubated at 37°C for the duration of one rotation. This period of time at a sampling rate of 120 tests/hour would be 20 minutes if all forty cups were used. However, the first 4 cups must be used for wash procedures which reduce incubation to 18 minutes. Synchronization of the

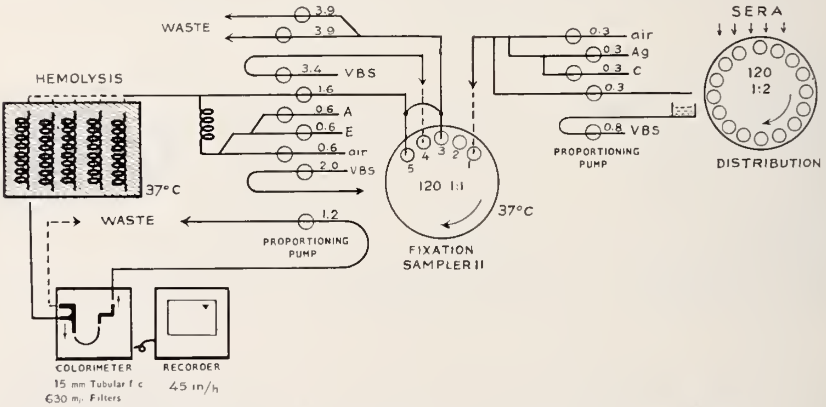
WASTE
3.9
3.9
3.4 VBS
HEMOLYSIS
1.6
0.6 A
0.6 E
0.6 air
2.0 VBS
37°C
WASTE
1.2
PROPORTIONING PUMP
5
4
3
2
1
120 1:1
37°C
FIXATION SAMPLER II
0.3 air
0.3 Ag
0.3 C
0.3
0.8 VBS
PROPORTIONING PUMP
SERA
120 1:2
DISTRIBUTION
COLORIMETER
15 mm Tubular f c
630 mμ Filters
RECORDER
45 in/h

FIG. 8-5: *Automation of Complement Fixation: Automatic Dilution Method* (see text). Note: Pump line size shown as flow rate in ml/min rather than tube diameter.

two samplers is achieved by adding diluted methylene blue to the first cup of the first sampler and initiating the second sampler just as this dye arrives.

On the second sampler, at the end of the incubation period, the complement fixation reaction cups are sampled into a reaction system of sensitized sheep red blood cells to determine their content of residual hemolytic complement. A system is designed to remove almost all of the contents of each cup and, following the sampling, the cup in its next position is filled automatically with VBS 2+ solution and, in its third post-sampling position, is emptied completely (83). This is accomplished with carefully positioned probes. Two sampling probes are spaced two cups apart, one samples the reaction for its residual hemolytic complement, while the other removes VBS wash solution. A fixed probe introduces the VBS wash solution into the middle cup following its repositioning after sampling. In this way, the entire instrument can be operated continuously because rinsed cups are suitable for reuse. The technician merely must place new samples in cups on the first sampler.

The chief disadvantage of this method is its short incubation for complement fixation. Another disadvantage is the problem of interpretation. In Figure 8-6, A is negative at 1:10 and 1:100, but B is positive at 1:10 and negative at 1:100. This result for B could be due to either antibody in B or very strong

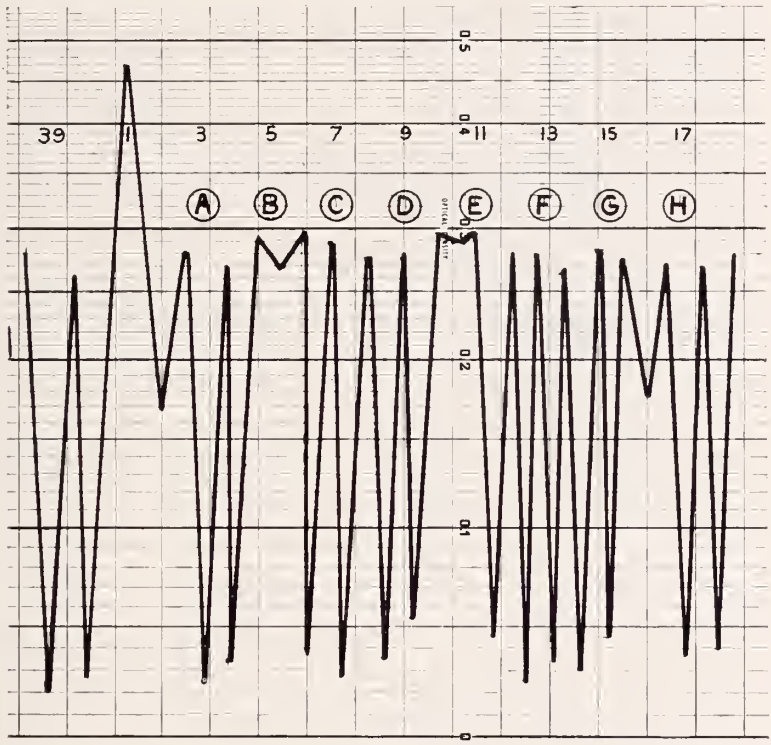

FIG. 8-6: *Graphs Obtained with Automatic Dilutions* (see text for explanation).

antibody in A that is active at 1:1000 but negative due to prozone at both 1:10 and 1:100. A second problem in interpretation arises from the presence of complement-fixing reactants in adjacent serum samples: e.g., Australia antigen and antibody to Australia antigen. Such reactants could support a positive reaction in a series of tests for totally unrelated antibodies such as antibodies to toxoplasma. Micromethods to detect antibodies which avoid the above defects have been and continue to be sought (82–84).

Acknowledgment

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NEUROLOGIC NOTES

JOHN LUSINS, M.D., EDITOR

Disseminated Idiopathic Inflammatory Histiocytosis Presenting Acutely as an Epidural Mass

Submitted by

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Introduction

The most common central nervous system pathology in histiocytosis X is caused by isolated damage to the pituitary gland or hypothalamus (1). About one in ten cases exhibits the Hand-Schüller-Christian triad of calvarial defects, exophthalmos, and diabetes insipidus; but a higher percentage develop just the endocrinopathy (2). Elian et al (3) recorded twenty-four cases of general xanthomatosis with neurologic manifestations resulting from subdural deposits or infiltration of the brain. One additional patient with involvement of the brainstem and cerebellum producing a posterior fossa syndrome was described by Beard and associates (4). The purpose of this paper is to report a unique case of idiopathic inflammatory histiocytosis that presented with neurologic symptoms and signs due to epidural compression.

Case Report

A twenty-five-year-old man came to the emergency room because of unremitting, severe, debilitating cephalgia confined to the occipital area. The patient described similar headaches during the previous three years, which were sharp in quality but brief in duration. He also noted hypersensitivity over the posterior aspect of his head and impaired sensation and clumsiness in his left arm and leg for two weeks prior to admission. Significant in his past medical history were two episodes of left otitis media at age eighteen and age twenty.

Palpation of the skull revealed diffuse swelling and tenderness of the occipitoparietal scalp and a calvarial defect in the right occiput about 3 cm in diameter. Otoscopic inspection disclosed a loss of normal landmarks in the left tympanic membrane. Neurological testing demonstrated a left hemiparesis, a left hemisensory loss, and a left Babinski's sign.

The cerebrospinal fluid obtained on admission and on the second and fourth hospital days was under normal pressure; there were no cells, the protein and sugar content were in the normal range, and cultures for bacteria and fungi were negative. Complete blood count, chest x-ray, electrocardiogram, serum chemistries, and urinalysis were all within normal limits. Three attempts to tap bone marrow from the iliac crest failed; bone marrow from the sternum was reported as normal.

Skull x-rays revealed large areas of irregular demineralization involving both the inner and outer tables (Fig. 1); the confluence of osteolytic lesions in the occipital and parietal bones

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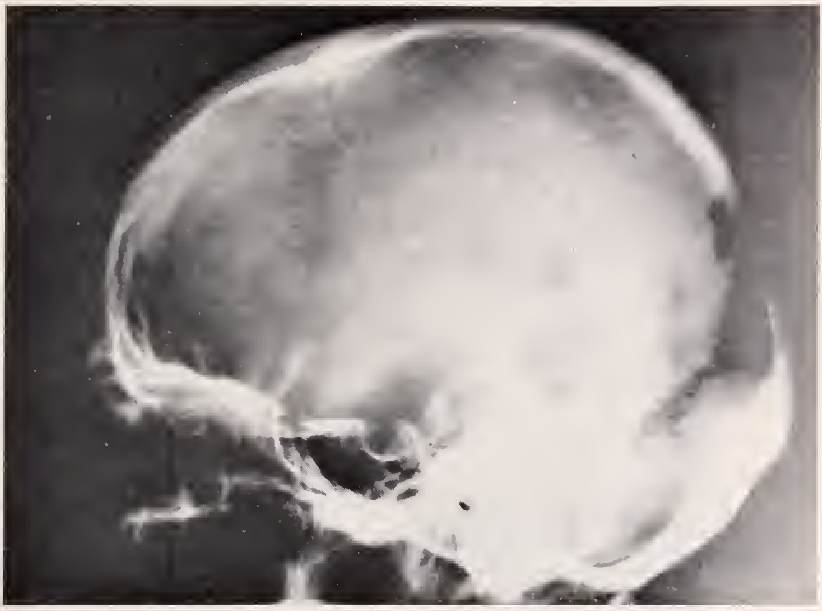

FIG. 1. Preoperative lateral view of skull, erosion of full thickness of calvarial vault.

formed a map-like pattern (Fig. 2). Additional destruction was noted in films of the pelvis (Fig. 3). Brain scan and electroencephalogram were interpreted as normal. Bilateral carotid and brachial angiography, including subtraction studies, failed to demonstrate any intracerebral mass lesion, vessel shifts, abnormal vascularity, or vascular occlusion; only a soft tissue mass eroding the calvarium in the occipitoparietal area could be identified.

The patient was begun on a course of steroid therapy which continued for ten days. Biopsy of the right occiput disclosed firm, grey, neoplastic tissue replacing the full thickness of bone, but confined to the epidural space. There was no involvement of the dura mater.

Sections of the biopsy material showed an inflammatory infiltrate within the bony defect consisting of a background of large pale-staining histiocytic cells; some histiocytes had multinucleation and vacuolation of the cytoplasm (Fig. 4, 5, 6). Scattered foci of eosinophils and occasional lymphocytes were present. The stroma appeared fibrous in some areas, but no significant reactive new bone formation was noted. Special staining for iron revealed focal accumulations of hemosiderin throughout the biopsy material.

Electron microscopy was performed on osmium-fixed biopsy material (Fig. 7). Histiocytic cells were identified with numerous infoldings of the cell membrane, lysosomes, and lipid bodies in the cytoplasm, and occasional groups of rod-like Langerhans cell granules (5, 6). Membranous complexes like those described by Basset and others (7) were not seen.

Initial radiotherapy consisted of 1200 rads to the skull and 1200 rads to the pelvis. The neurologic symptoms and signs disappeared over a three-month period. Subsequent lesions in the left mastoid process, right scapula, and lower thoracic vertebrae were treated with sequential doses of 600 rads. One year after hospitalization, his chest x-ray showed the honeycomb pattern characteristic of disseminated idiopathic inflammatory histiocytosis (Fig. 8). Two years after the diagnosis was made, the patient remains asymptomatic.

Discussion

The neuropathology seen in histiocytosis X has been explained by lesions of the sellar area compressing the hypophysis or hypothalamic nuclei and calvarial

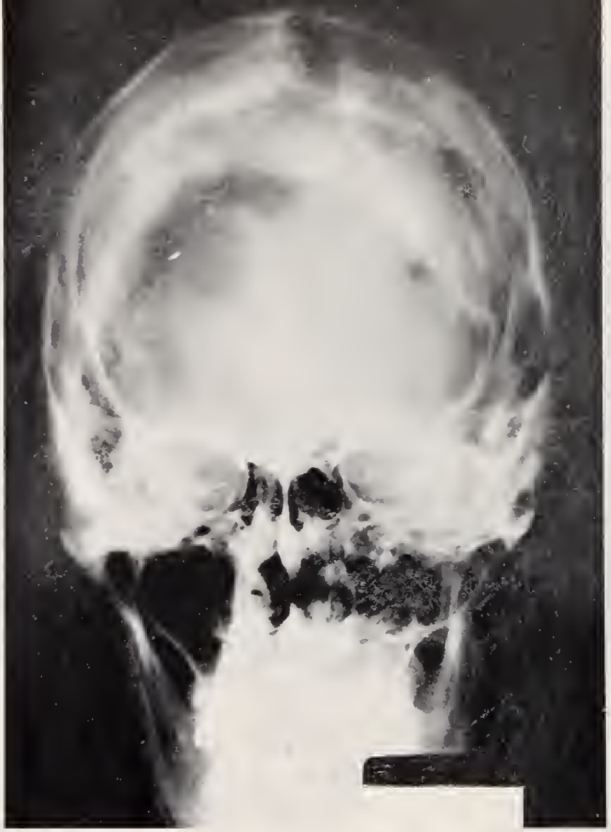

Fig. 2. Postoperative Towne view of skull, geographic pattern of osteolytic lesions. Note metal clip identifying area of biopsy.

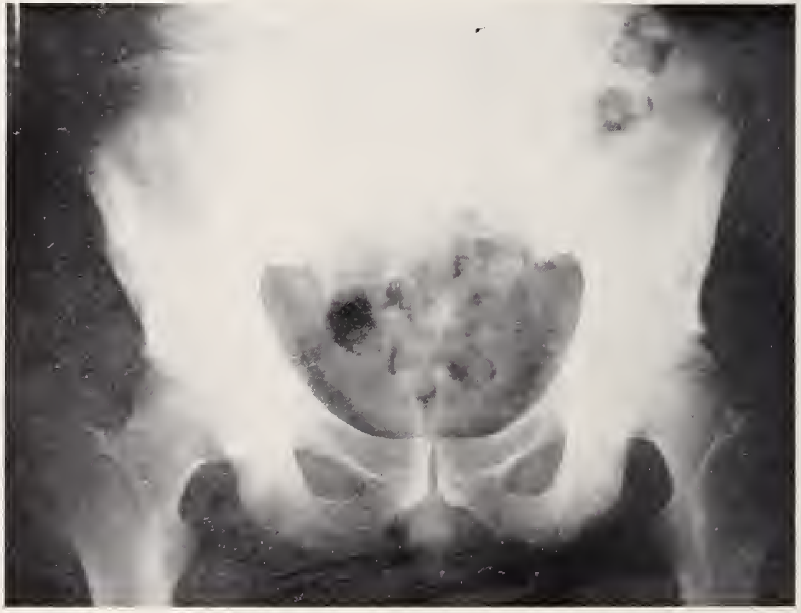

Fig. 3. X-ray of pelvis, marked reactive sclerosis in both iliac bones, indicating healing phase.

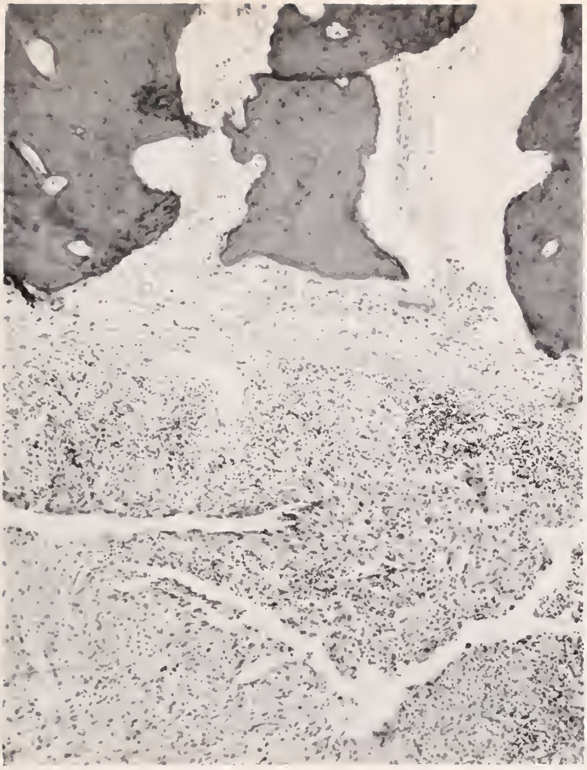

FIG. 4. Pleomorphic cellular infiltrate filling a bony defect in the calvarium. No reactive osteogenesis is seen at the periphery of the lesion (H & E × 60).

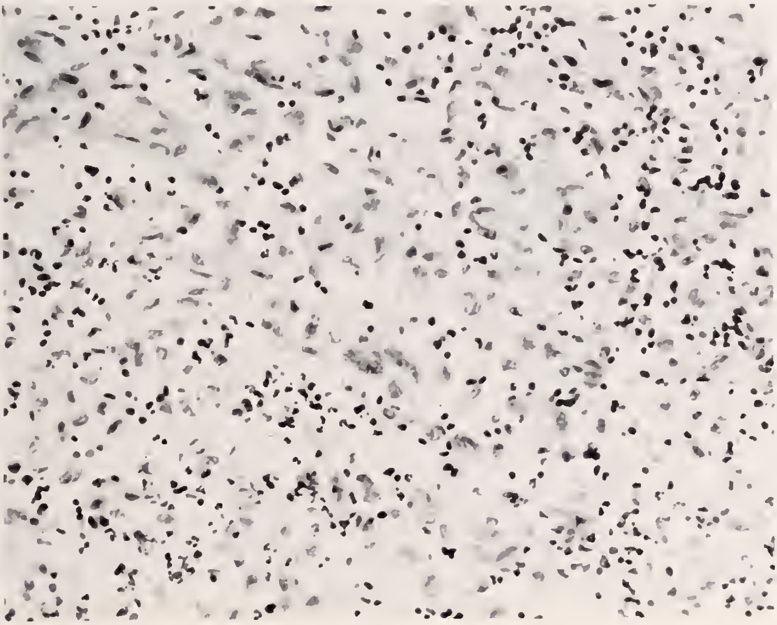

FIG. 5. Idiopathic inflammatory histiocytosis, characterized by a richly cellular infiltrate composed predominantly of pale-staining histiocytes, some fibroblasts, and groups of eosinophils (H & E × 320).

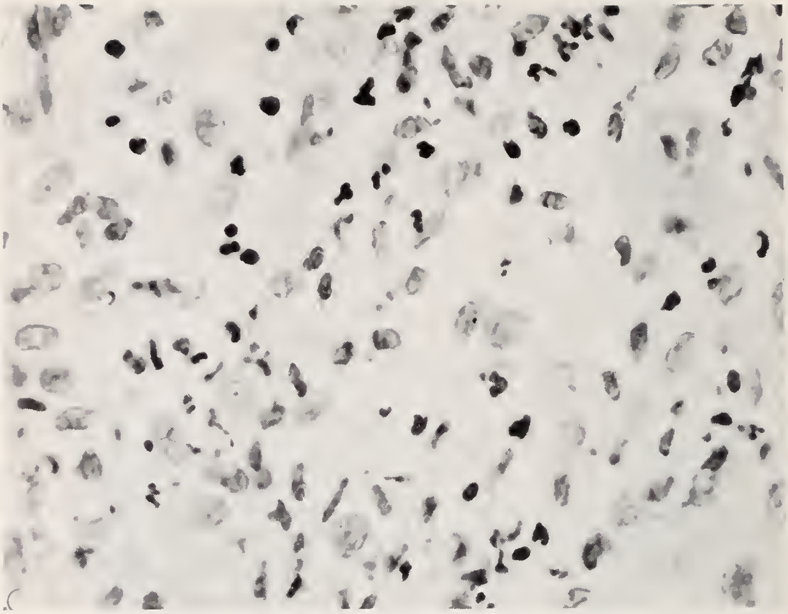

Fig. 6. High power view of multinucleated histiocytes with pale vacuolated cytoplasm (H & E × 800).

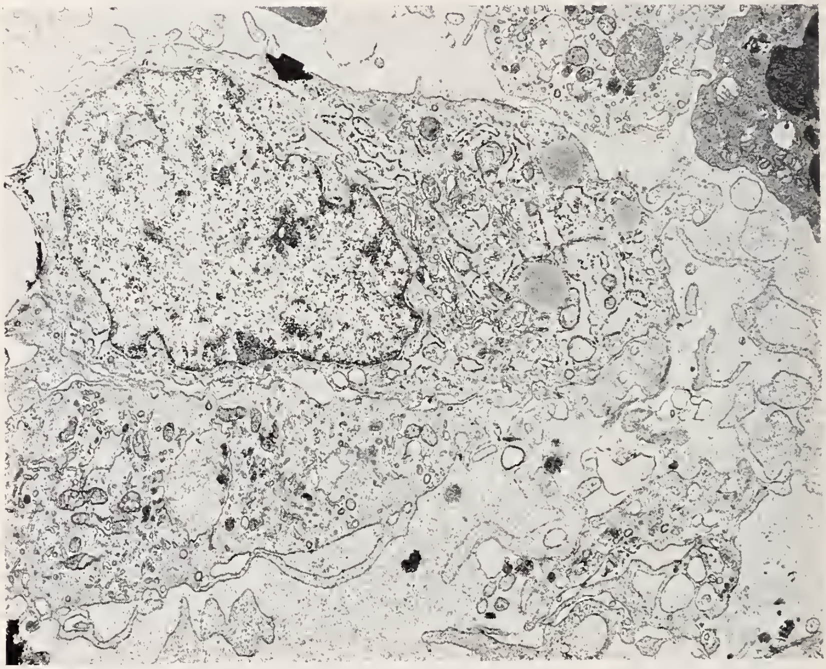

Fig. 7. Electron micrograph of histiocyte, showing rod-shaped Langerhans cell granules in the cytoplasm (arrow). Other rod-shaped structures can be seen to the right of the nucleus in the Golgi apparatus. The rods have an axial filament (× 5000).

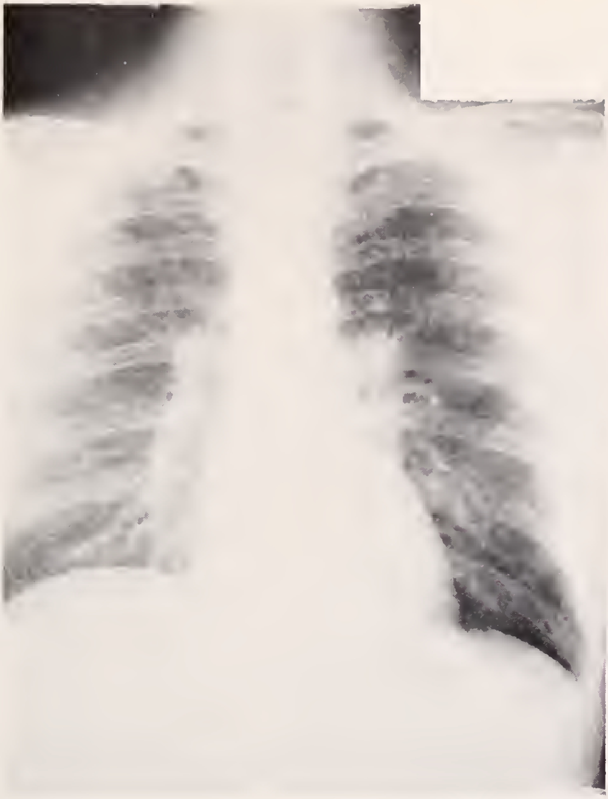

FIG. 8. Chest x-ray, honeycomb appearance of diffuse interstitial fibrosis.

lesions infiltrating the dura and underlying cerebral parenchyma. Neoplastic proliferation of the reticuloendothelial system may originate in the neural tissue itself from perivascular foci of adventitial histiocytic elements (8). In our own case, an epidural focus produced transient hemispheric compression without actual infiltration of the meninges and apparently resolved by spontaneous decompression of the skull and hemorrhagic necrosis.

Summary

A variety of neurologic symptoms, resulting from subdural deposits or infiltration of the brain and generally appearing in the late stages of Hand-Schüller-Christian disease, have been described in twenty-five cases of histiocytosis X in the world literature. An unusual case is reported of a twenty-five-year-old man with disseminated idiopathic inflammatory histiocytosis that presented acutely as an epidural mass. The diagnosis was made initially by x-ray findings and confirmed by surgical biopsy.

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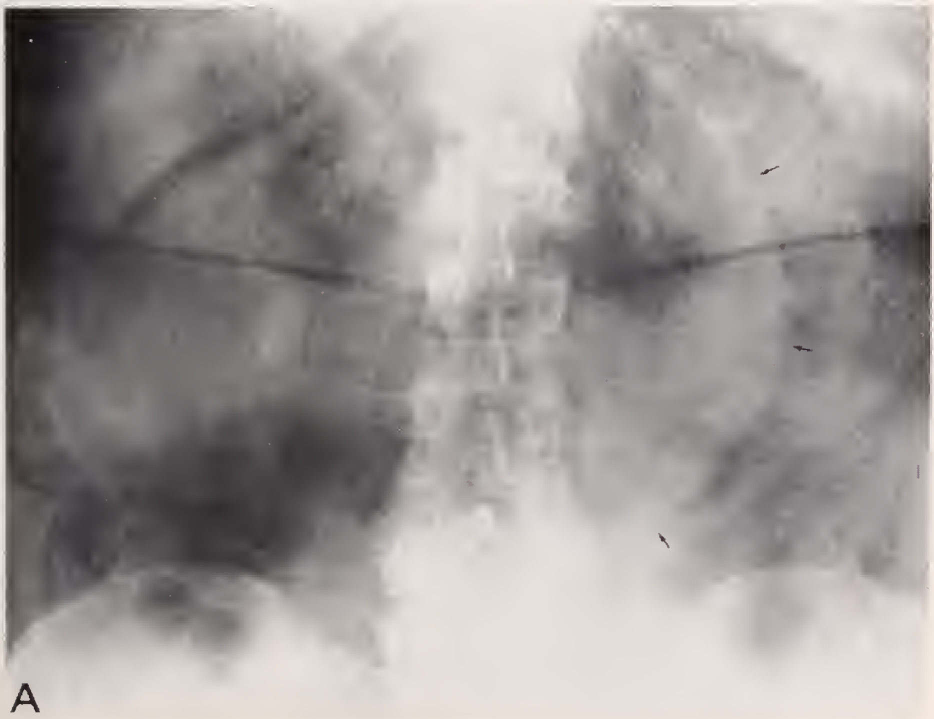
RADIOLOGIC NOTES

CLAUDE BLOCH, M.D. AND HARVEY M. PECK, M.D., COEDITORS

CASE NO. 378

This 55-year-old woman was admitted with a seven-month history of nausea, epigastric fullness, and belching. The patient had only one episode of melena. Physical examination revealed an obese woman with normal vital signs. A tender, grapefruit-sized epigastric mass was noted. Blood count was normal and the stool guaiac was negative. Radiographic examination of the abdomen in the supine projection revealed a well-demarcated, centrally located large epigastric mass (Fig. 1A, along arrows). Although the center of the mass appeared less dense than would be expected for a lesion of this size, its true lucent center was seen to better advantage in the lateral view (Fig. 1B). Barium meal examination



Case 378, Fig. 1A: Supine film of the abdomen reveals a large epigastric mass. Its left lateral contour is well demarcated (along arrows) and is sharply outlined. The center of the tumor, just to the left and the right of the spine, appears lucent.

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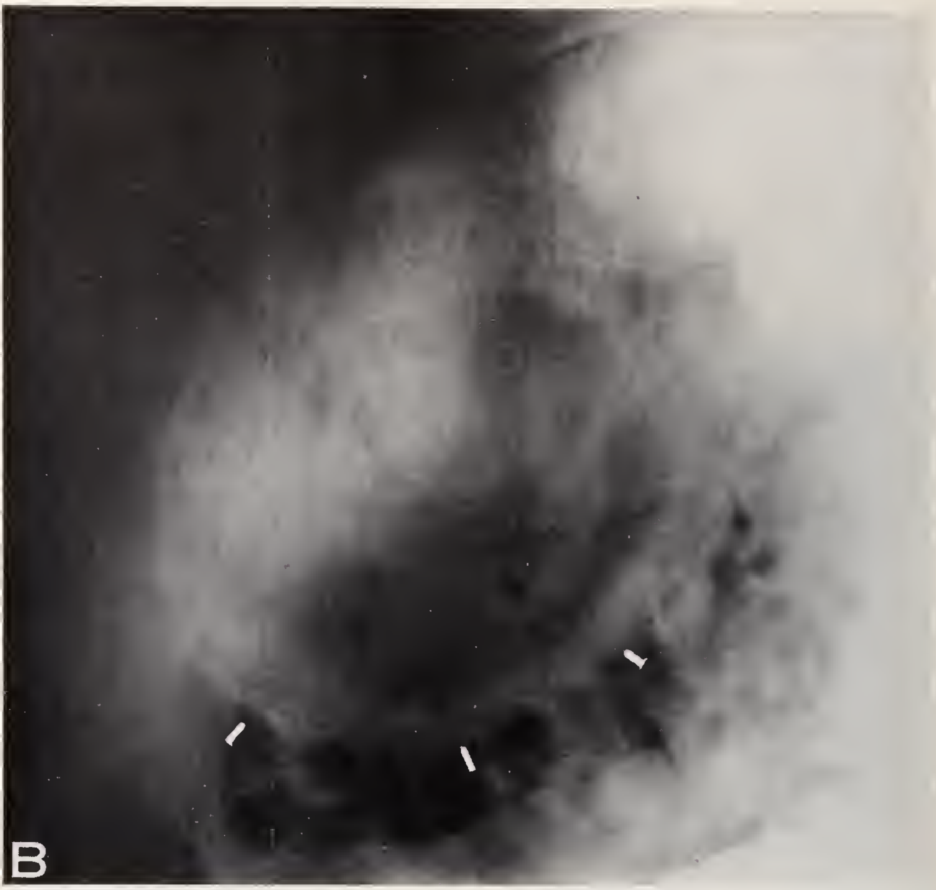
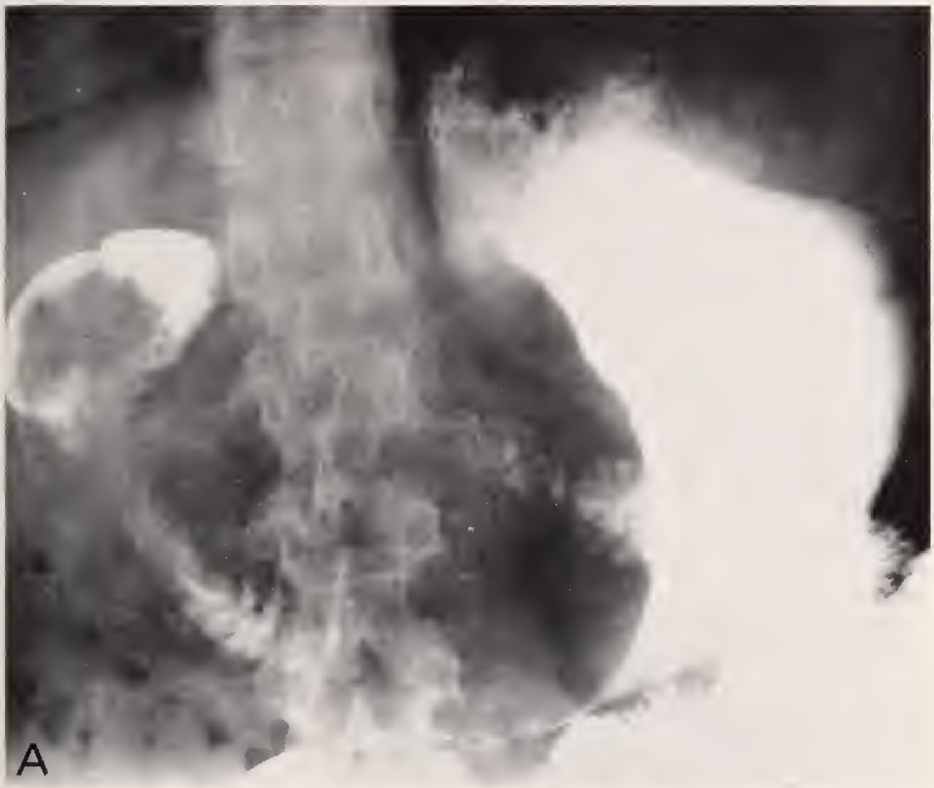


Case 378, Fig. 1B: Right lateral view of the abdomen demonstrates the mass to better advantage. Its lower margin and thick wall are well seen (along arrows). The lucent fat-containing center is clearly visible.

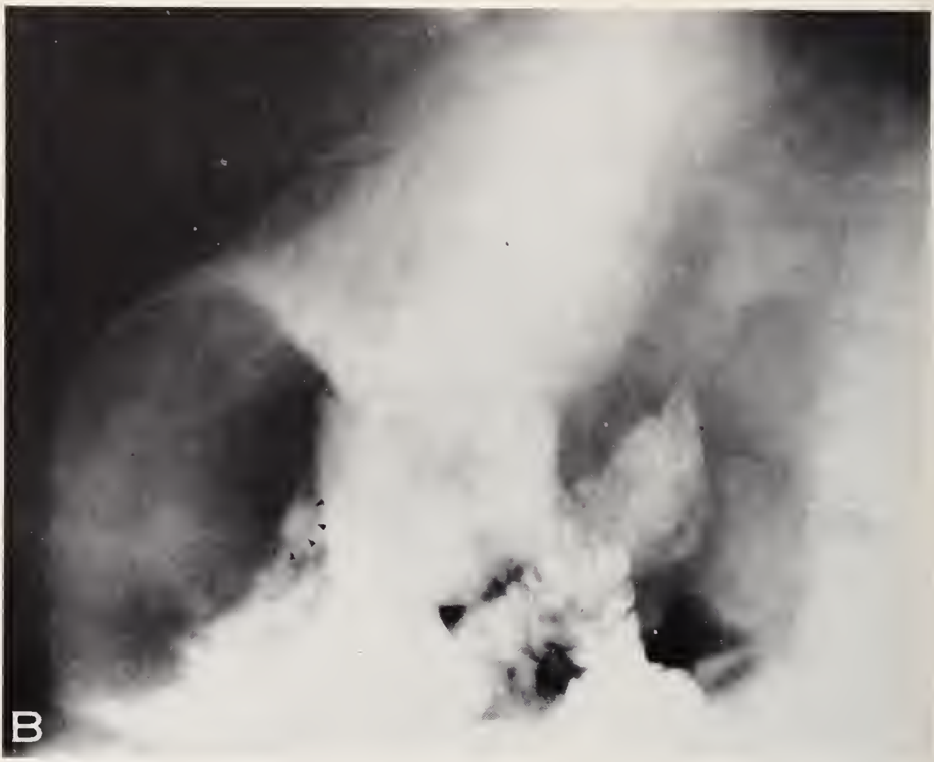
demonstrated a huge, sharply demarcated mass occupying the distal two-thirds of the stomach (Fig. 2A). In the lateral view, it was seen as a sessile mass with sharp borders arising from the anterior wall of the stomach (Fig. 2B). An amorphous 2 × 3 cm in diameter barium patch near the apex of the mass represented a shallow ulceration (along arrows). At laparotomy, a huge cystic submucosal lipoma was found arising from the anterior gastric wall. It extended distally to the pylorus and proximally to the upper third of the stomach. In its midportion, there was a 2 × 3 cm shallow ulceration.

Discussion

Gastric lipomas are rare, with only 103 cases reported prior to 1961 (1). They represent only 3% of all benign gastric tumors (2). Most of the cases described in the literature are small; giant gastric lipomas, such as the one described



Case 378, Fig. 2A: Barium meal examination in the posteroanterior view demonstrates the proximal extent of the gastric mass, as well as its sharp delineation.



Case 378, Fig. 2B: In the right lateral view, barium outlines its sessile base along the anterior gastric wall. A small shallow ulceration is seen at the apex of the mass (along arrows).

here, are exceedingly rare. The symptoms produced by gastric lipomas can usually be correlated to their size and the presence of overlying mucosal ulcerations. 75% of them arise in the antrum and 90% present as a submucosal mass. Only 10% have an exogastric component (3). Half of the patients have signs of hemorrhage, one-third present with abdominal pains, and 20% have obstructive symptoms (4). Sarcomatous degeneration is rare.

Case Report: GIANT GASTRIC LIPOMA.

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Acknowledgment

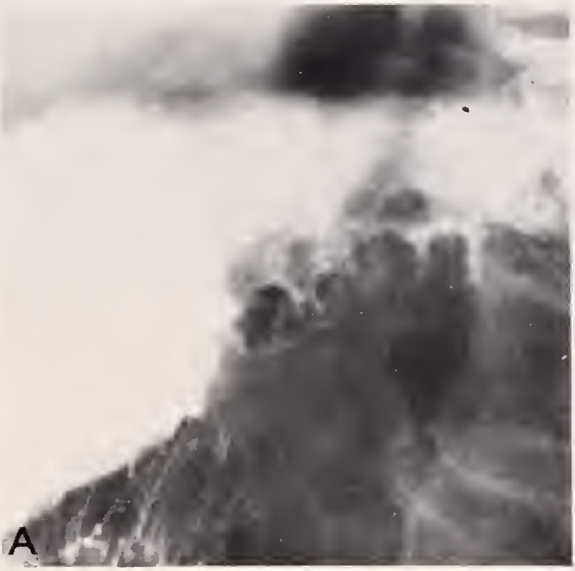
The editors wish to thank Drs. Maurice J. Zimmerman, Martin J. Wiener, and Isadore Kreel for their permission to publish this case.

CASE NO. 379

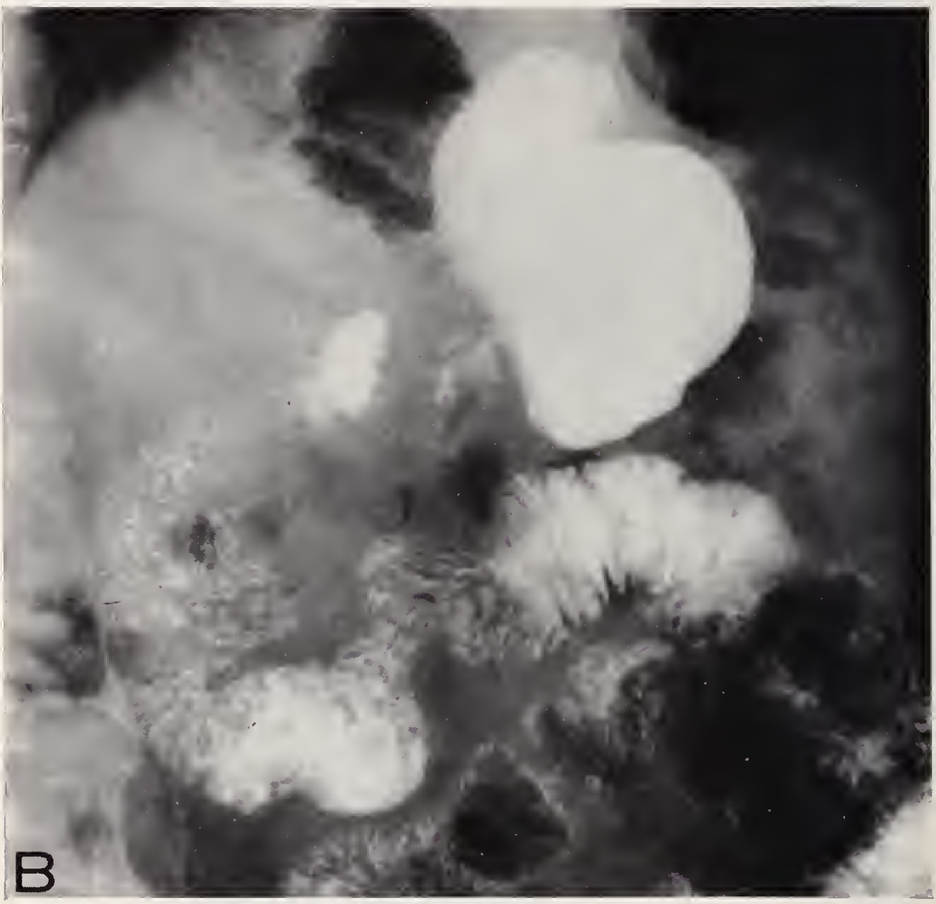
This 75-year-old man was evaluated because of weight loss, recent instability in a long-existing diabetic condition, and moderate anorexia. Physical examination demonstrated no significant abnormalities. No abdominal masses could be palpated. Hemogram and urinalysis were normal except for glycosuria. X-rays of the kidney and chest were normal. Gastrointestinal series showed fixation of a limited portion of the posterior wall of the stomach (Fig. 1A). The folds were thickened and nodular, but there was no evidence of ulcerations or intraluminal mass. There was also slight downward displacement of the gastro-duodenal junction, where the folds were pleated and fixed (Fig. 1B, arrows). Barium enema revealed invasion of the medial wall of the proximal descending colon with a nodular, fixed mucosa over a short segment of the bowel (Fig. 2, arrows). There was no ulceration or intraluminal mass, so that the findings were quite similar to those in the stomach. Laparotomy showed an inoperable carcinoma of the tail and distal portion of the body of the pancreas with local invasion of the stomach, colon and spleen.

Discussion

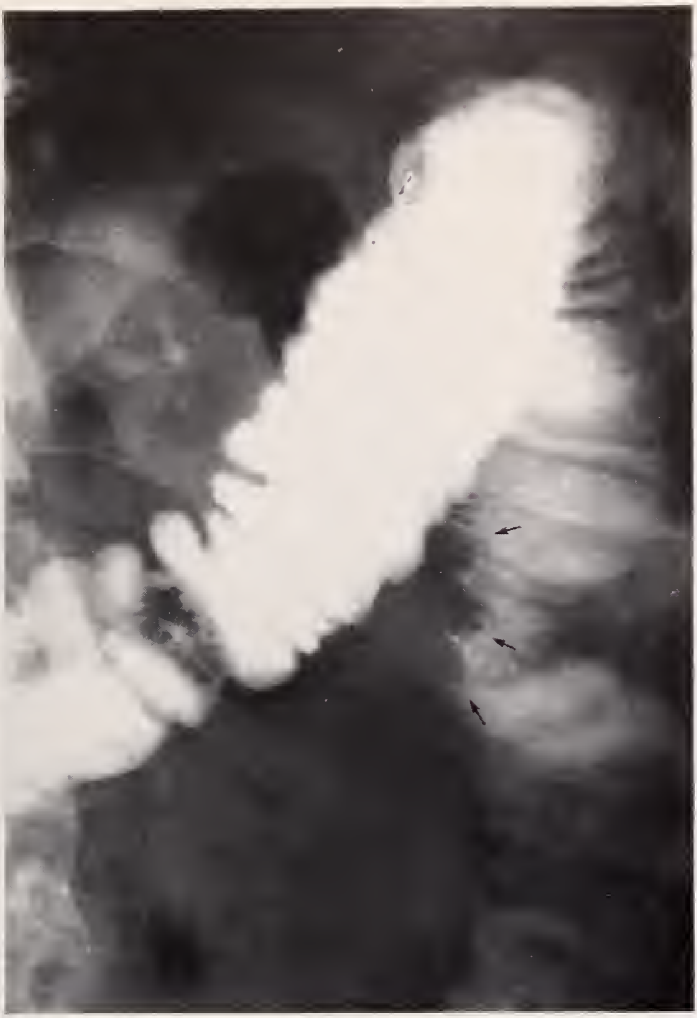
The roentgen demonstration of the localized area of invasion of the stomach is often the only evidence of a carcinoma of the distal portion of the pancreas. As in the case presented here, a small segment of rugal folds along the posterior wall of the greater curvature of the stomach was involved; this subtle finding



Case 379, Fig. 1A: Pressure spot film of the barium-filled stomach in the left anterior oblique view reveals nodular fixed gastric mucosa along the posterior wall of the greater curvature. This is only seen over a limited segment of the stomach, adjacent to the tail of the pancreas. No ulceration or intraluminal mass is noted.



Case 379, Fig. 1B: In the right anterior oblique view, there is slight depression of the gastro-duodenal junction, with pleating of the folds. The mucosal folds, although not ulcerated, are fixed by the local tumor invasion.



Case 379, Fig. 2: The descending limb of the splenic flexure, on its medial wall, demonstrates a local infiltration of the folds with nodularity and distortion of the involved segment (arrows). No ulceration or mass lesion can be outlined.

underlines the importance of impeccable technique and meticulous study of the gastrointestinal series in the absence of a gross mass lesion or ulceration. The nodular appearance of the folds, both in the stomach and splenic flexure, is due to tumor invasion of the serosa and submucosa with fixation of the mucosa itself. In the erect barium-filled stomach, the cascaded fundus is unable to "reduce" itself. This, too, is often evidence of a mass or local infiltration along the posterior wall of the stomach. Localized invasion of the ligament of Treitz, often with only fixation (pleating) of the folds and no displacement, is a well-known sign of carcinoma of the distal pancreas.

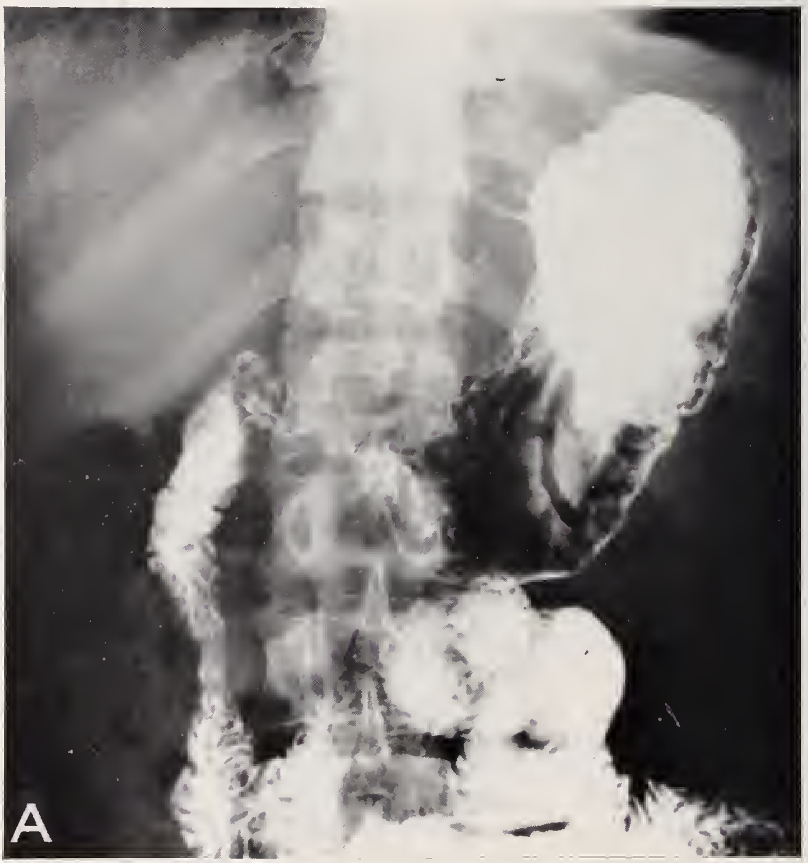
Case Report: CARCINOMA OF THE DISTAL PANCREAS WITH LOCAL INVASION.

Acknowledgment

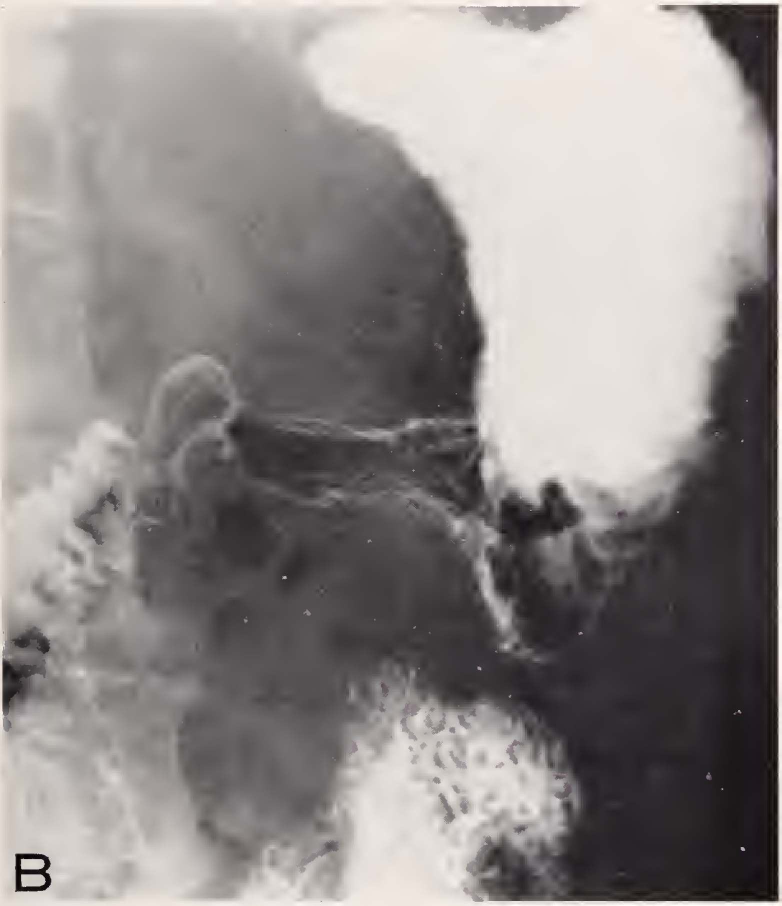
The editors wish to thank Dr. James Vogel for permission to publish this case.

CASE NO. 380

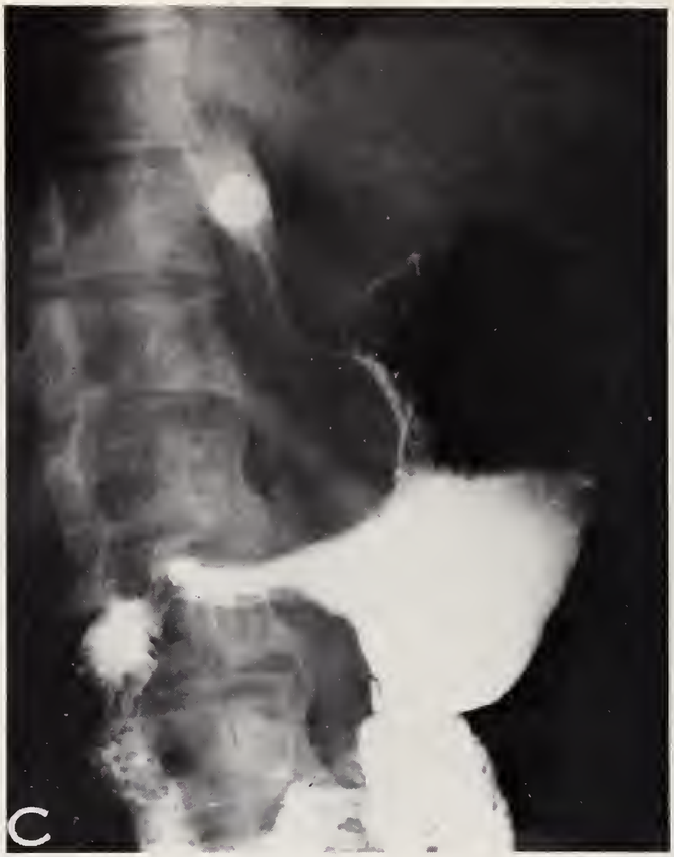
This 55-year-old woman had undergone left radical mastectomy seven years previously. Pelvic bone metastases, first noted three years before, responded well to androgen therapy. Two years before the patient was given chemotherapy for skin and liver metastases. A marked iron deficiency anemia had been noted for



Case 380, Fig. 1A: Supine film of the barium-filled stomach reveals a narrowing of the distal third of the stomach. No ulceration is demonstrated. There is evidence of a large left pleural effusion. The bones demonstrate diffuse metastatic disease.



Case 380, Fig. 1B: In the left posterior oblique projection, with air outlining the gastric antrum, there is a large shallow contour defect due to rigidity and infiltration of the greater curvature.



Case 380, Fig. 1C: In the erect right anterior oblique projection, the extent of the infiltrative process is seen to better advantage. There is shortening of the entire lesser curvature, and there is circumferential involvement of the distal third of the stomach. No ulcerations or intraluminal masses are seen.

the last three years. The stool guaiac was positive and the hematocrit ranged between 25% and 35%. Physical examination revealed a thin, chronically ill-appearing female with evidence of widespread metastases to the liver, skin, and right breast. Gastrointestinal series showed extensive infiltration of the stomach, with restricted distensibility of its distal third (Fig. 1). There was no intraluminal mass or ulceration. The antrum was narrow with rigidity along its greater curvature. The entire lesser curvature was shortened and distensibility reduced. An extensive left pleural effusion and bony metastases could be noted.

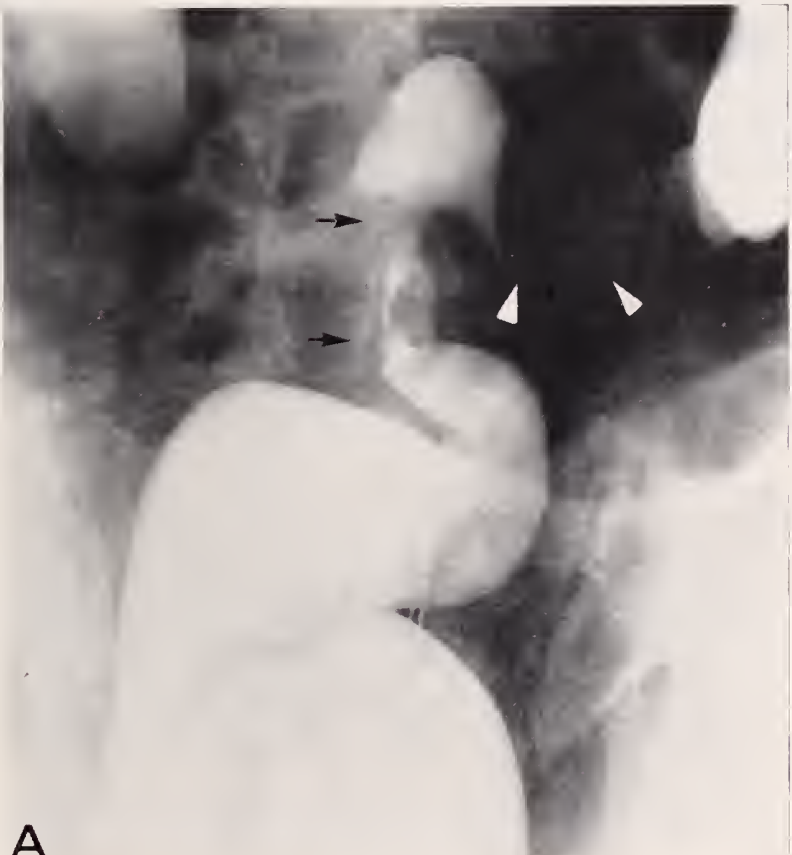
Case Report: METASTATIC CARCINOMA OF THE BREAST TO THE STOMACH.

Acknowledgment

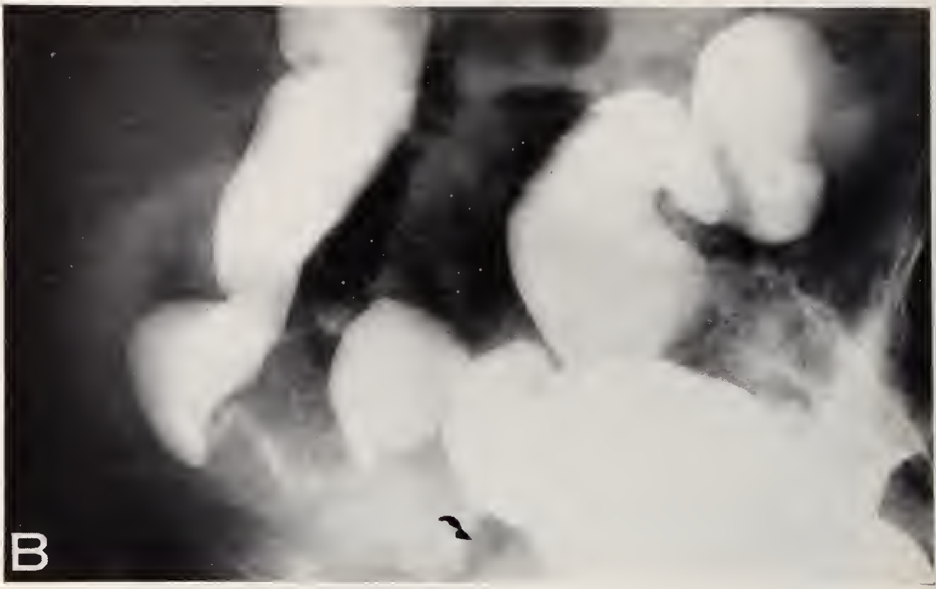
The editors wish to thank Dr. Lynn H. Ratner for permission to publish this case.

CASE NO. 381

This 66-year-old patient had a left radical mastectomy six years before and now presented with signs and symptoms of advanced metastatic carcinoma of the breast. Physical examination of the abdomen revealed palpable pelvic and left lower quadrant nontender, hard masses. The patient was anemic, but had guaiac negative stools. Barium enema revealed two separate lesions in the sigmoid. The one in the distal sigmoid was circumferential, with a narrowed, slightly eccentric lumen (Fig. 1 A, black arrows), and the second in the proximal sigmoid colon presented as a smooth, broad contour defect along the superior wall of the bowel (Fig. 1 C, arrows). Numerous bony metastases could be seen.



Case 381, Fig. 1A: Angled view of the barium-filled sigmoid reveals two separate lesions. The distal one is circumferential (black arrows) with a narrow eccentric lumen, but no ulceration or true overhanging edges. The second lesion is within the proximal sigmoid (white arrows) and presents as a smooth, round contour defect along the superior wall of the colon. No ulcerations are seen.



Case 381, Fig. 1B: In the oblique view, the proximal lesion is noted to be causing narrowing of the lumen, but there is no evidence of proximal dilatation.

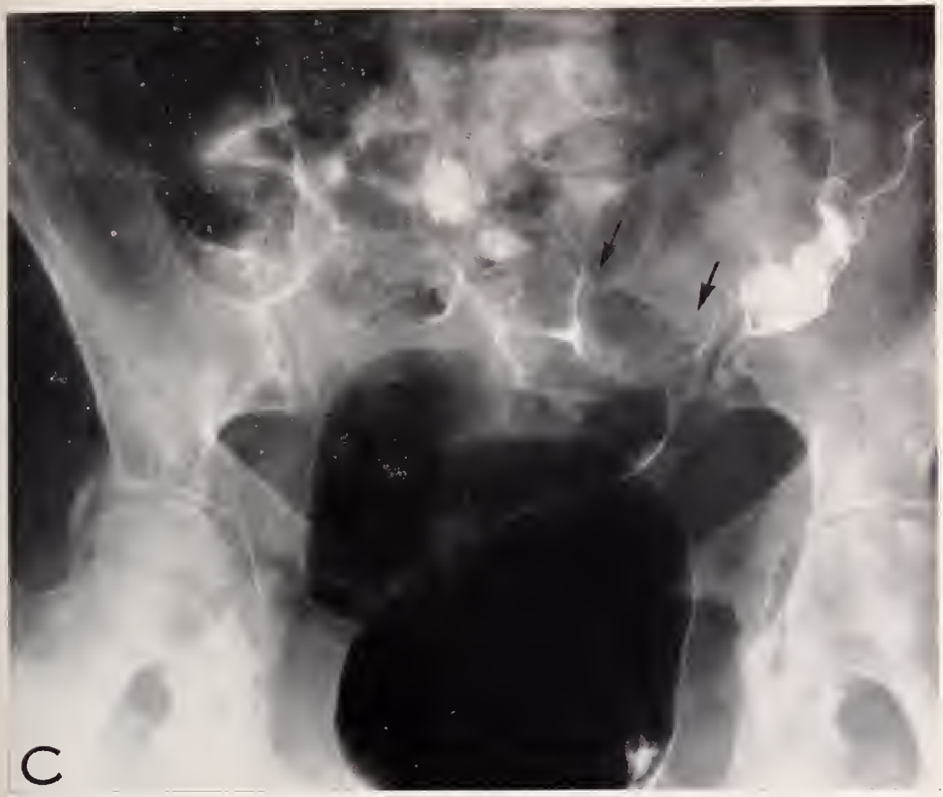
The patient was placed on chemotherapy and discharged on maintenance therapy.

Case Report: Metastatic carcinoma of the breast to the colon.

Discussion of Cases 380 & 381

Metastatic carcinoma of the breast to the stomach is usually part of advanced generalized spread of the disease. Although poorly recognized and infrequently reported until recently (1), this entity is being recognized with increasing frequency. Radiologically, it is often indistinguishable from primary scirrhous carcinoma of the stomach. As was pointed out in our previously reported cases (2), gastrointestinal metastases appear to occur more frequently in patients who have received steroid therapy during the course of their treatment. The patient presented here, however, did not receive any corticosteroids for her metastatic treatment.

The most common primary carcinomas to metastasize to the colon are carcinomas of the stomach, pancreas and ovary (by direct extension), and breast, lung and kidney (by hematogenous spread). Metastatic involvement of the colon is very rare, and when it occurs it is usually part of an advanced stage of the disease. The colonic lesions are usually multiple and present as broadbased contour defects with an intact mucosa. It is rare for the mural metastasis to appear as a circumferential lesion, such as was seen in the distal sigmoid of Case No. 381.



Case 381, Fig. 1C: Air contrast study shows the proximal lesion to be smooth in contour and intraluminal in position (along arrows) with an intact overlying mucosa.

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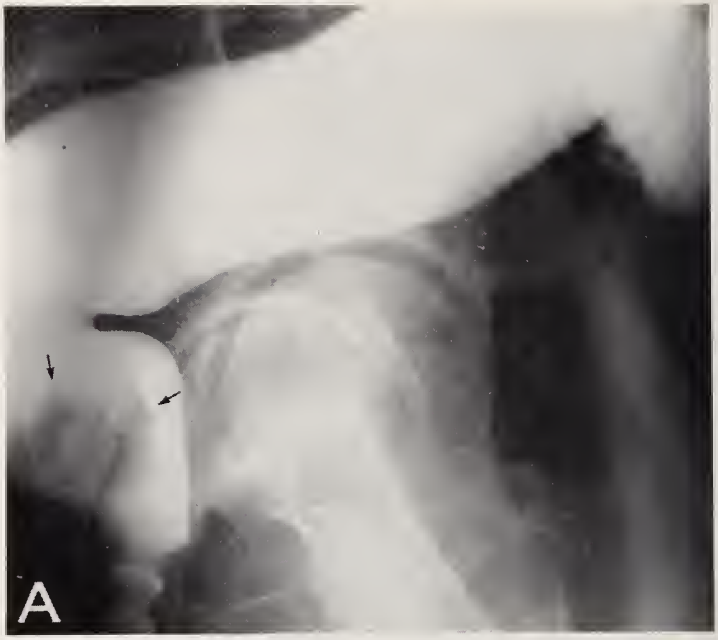
CASE NO. 382

Submitted by

JACOB LICHY, M.D.

This 75-year-old man was hospitalized because of a three-months' history of bright red rectal bleeding. Twenty years before, at the time of hemorrhoidectomy, a pigmented growth near the rectum was resected. Histologically, this was a malignant melanoma. Two years later, a right inguinal mass was noted

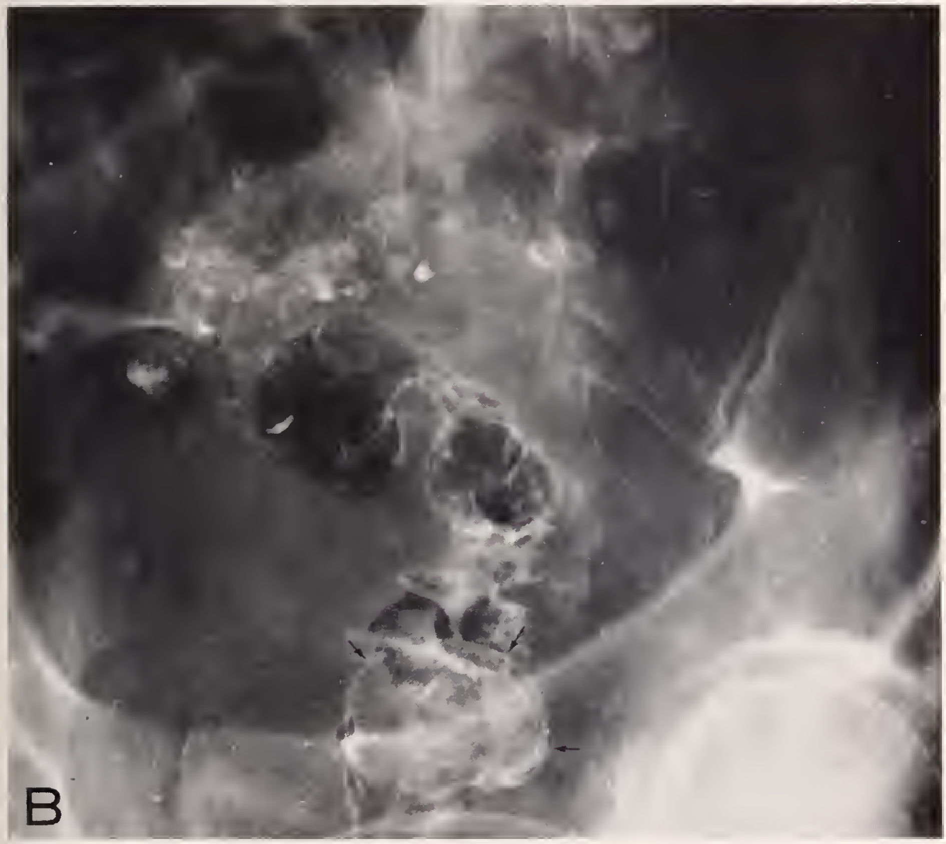


Case 382, Fig. 1A: Lateral view of the barium-filled rectum and sigmoid reveals a lobulated sessile mass arising from the posterior wall of the rectum, just above the anal verge (along arrows). The mucosa is intact over the lesion. The walls of the rectum, immediately adjoining the mass, are normally distensible.

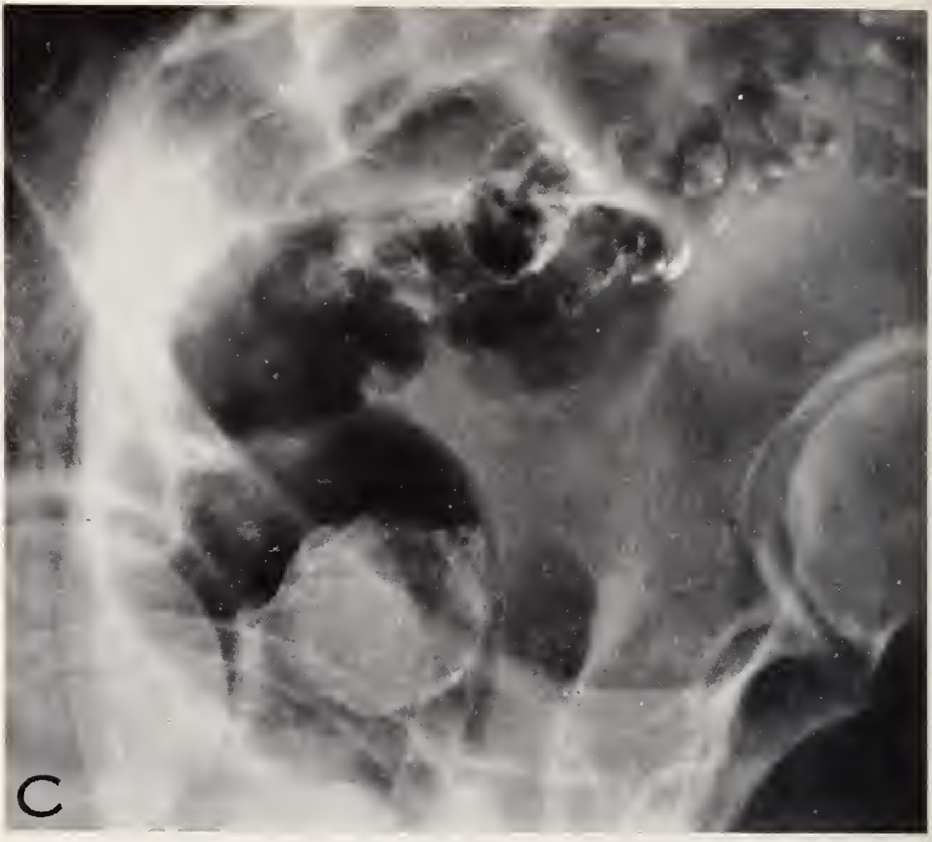
and a unilateral groin dissection was performed; this was also malignant melanoma. Three years before the present admission, namely 17 years after the original diagnosis, a left inguinal mass was found which had the same histology. Two years before, a rectal polyp was removed that was a melanoma. One year before, the patient was placed on chemotherapy for metastatic disease. At the time of the present admission, the patient had masses in the anterior chest wall, right side of the pelvis and epigastrium. Rectal examination and proctoscopy revealed a bulky polypoid mass arising from the posterior wall of the rectum, just above the anal verge. Biopsy of this lesion was malignant melanoma. Barium enema revealed a sessile, lobulated bulky mass in the rectum along its posterior wall (Fig. 1A, arrows). No ulcerations were seen along its surface and the surrounding rectal walls were normally distensible (Fig. 1B & 1C). The patient's rectal bleeding ceased after local excision.

Discussion

Rectal melanomas are rare and their prognosis is usually very poor. They are spread by the local lymphatics to the inguinal regions and later by hematogenous routes. Patients usually first experience bleeding (1) or local pelvic or perineal pain. Often the lesion is discovered accidentally when the patients present themselves for "hemorrhoid" operation. Not all rectal mela-



Case 382, Fig. 1B: The smooth upper margin (along arrows) is well demonstrated on the post-evacuation film.



Case 382, Fig. 1C: Air contrast study in the right anterior oblique view shows the lobulated surface of the mass with its broad base inferiorly.

nomas are pigmented, so that grossly they can be mistaken for hemorrhoids or skin tags. Some bulkier tumors, as in the case presented here, infiltrate up to and cross the dentate line, presenting as rectal lesions (2). Radiologically, one must differentiate this lesion from the more common villous tumors and the polypoid carcinomas.

Case Report: MALIGNANT MELANOMA OF THE RECTUM

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EDITORIAL

The occasion of the fortieth anniversary of the Journal and the change in editorship afford the opportunity both for *recherche du temps perdu* and for restatement of aims and policy.

The Journal was founded in 1934 largely through the energies of Dr. Joseph H. Globus, a man of great charisma and academic stature. Dr. Globus, a pioneer in neuropathology, clearly stated the aims and policies of *The Journal of The Mount Sinai Hospital* in the following foreword reprinted from Volume 1, Number 1.

FOREWORD

> *The Mount Sinai Hospital after some deliberation decided to publish a journal devoted principally to case reports.*
>
> *This decision was prompted by the realization that important leads have often been obtained by a careful observer from significant findings in a single case and many advances in clinical medicine have had their inception in such studies. Thus it was felt that a real need exists for a publication contributing to casuistic medical literature.*
>
> *A departure from the policy of including only case reports will be made from time to time by the publication of the William Henry Welch, Edward Gamalid Janeway and other lectures, which are delivered annually at The Mount Sinai Hospital by prominent clinicians and outstanding investigators.*
>
> *This is the plan to which the editorial board of the JOURNAL OF THE MOUNT SINAI HOSPITAL is committed at present. Whether it shall remain as a permanent policy of the JOURNAL will depend upon the views expressed by friends of the hospital and its JOURNAL, whose frank criticism is invited.*
>
> *The editorial board takes this opportunity to thank the Trustees of The Mount Sinai Hospital for their moral and financial support which made this undertaking possible.*

Restriction to case reports did not long persist. During the 20-year editorship of Dr. Globus, the Journal became a general medical publication within which appeared a surprising number of classic reports. This metamorphosis is elegantly expressed in an editorial by Dr. Lester Tuchman in 1954 when he became Editor. The editorial reprinted below reflects the wide interests of the new Editor, Dr. Lester Tuchman, a fine clinician, investigator, educator, and later an expert on the socioeconomic aspects of the delivery of medical care.

(From *The Journal of The Mount Sinai Hospital,*
Volume 21, Number 1, May–June 1954)

EDITORIAL

Twenty years ago the late Joseph Globus wrote the opening editorial for the Journal of the Mount Sinai Hospital, setting it in the path it followed until his death two years ago. The brief tenure of Solon Bernstein interrupted by his illness and terminated so abruptly by his untimely death saw no change in these policies.

It is now the duty of the new Editor and the Editarial Board to reexamine the purposes of the Journal, and either to reaffirm the old aims or fix new ones.

We believe that a major aim of the Journal, now as then, is to make available its pages for case reports based upon the extraordinary clinical material which our Hospital has always had, some of which might otherwise be lost. Close observation, accurate, concise description, and detailed reporting are to be encouraged. We hope to foster the type of medical writings exemplified so brilliantly by men like Parkes Weber, and our own great Emanuel Libman. The Journal would prefer these papers to come from the ranks of the House and Resident Staffs, Fellows, and younger men generally. We are therefore offering an annual prize to be known as the Joseph H. Globus Memorial Prize for the best such paper published in the Journal during each calendar year. The prize may not be won by anyone of Associate or Attending rank and is open to all other Mount Sinai Hospital workers. The subject may pertain to any clinical aspect of medicine, surgery, or the other specialties, based upon material from the Mount Sinai Hospital.

At the other end of the spectrum we hope to publish from time to time fruits of the maturity, wisdom and experience of our Nestors, our Elder Statesmen, men who through their own medical lives and those of their teachers have encompassed all the rise of modern medicine—men who have themselves made and in most instances are continuing to make many of the contributions which have made our Hospital great. From them we hope for philosophy, for perspective, or prophecy, for new directional signposts, for approbation and admonition.

From the active workers of established position we desire no more than a tithe of their productivity.

The clinico-pathological conferences and the monthly conferences are rich mines ripe for exploitation, the many named lectures will continue to be reported whenever possible. Brief general reviews of newer expanding experimental fields by men who are making their own contributions within them will be welcomed.

Finally, we hope that the Journal will be representative of all the Hospital and every department, and that every worker, clinical or laboratory, will support it, read it, and write for it.

Under Dr. Tuchman's guidance, The Journal maintained its standard of excellence and its preeminence among journals published by hospital centers, major clinics, and medical schools. The going was not easy and the direction not always clear, especially with the enlargement of the Hospital to the Medical School and Center. The trials and tribulations are eloquently voiced in Dr. Tuchman's recent letter to Dr. Schaffner:

March 21, 1974

Fenton Schaffner, M.D.

Chairman, Committee on Medical
Education and Publications
The Mount Sinai Hospital
New York, N.Y. 10029

Dear Dr. Schaffner:

Having submitted my resignation as Editor of The Mount Sinai Journal of Medicine to the Committee on Medical Education and Publications, I think it appropriate to make some observations on the history of the Journal, to express some hopes for its future, and to make some suggestions for changes that need to be made.

An effective date of resignation on May 1st will mark for me five years as Assistant Editor and twenty years as Editor of The Journal of The Mount Sinai Hospital, now known as The Mount Sinai Journal of Medicine. The Journal has now completed forty years of existence. The name change was made to recognize that we are no longer the organ of one hospital, but the publication of a large medical school complex, including five hospitals, a medical school, a school of basic sciences, and several smaller loci of learning. Obviously, this expanded base presents greater opportunities—a potential not yet capitalized upon.

The Journal goes to over 300 medical school libraries, in this country and abroad. Foreign readership is impressive, as evidenced by the large numbers of reprint requests which come from scores of countries and from all continents. Members of our staff returning from the most remote and exotic countries, as well as from famous old centers, are continually astounded at the respect in which the Journal is held all over the world.

For continuing growth in excellence, Chiefs and Department Heads must be persuaded and must persuade members of their departments that the Journal is their Journal and merits their support. There follows a brief outline of some of the reasons the Journal should be supported as a general journal, bearing the imprimatur of the Hospital and the Medical Center and, for better or worse, the prestige of the institution.

1. A need exists in many areas for periodic syntheses. From time to time, isolated facts and findings of research require assembly, evaluation, and interpretation. The Journal is an ideal forum for such publications which would be warmly welcomed and well received, not only by confreres in related investigative fields, but also by interested clinicians.

2. Reviews, in our experience, are always popular and reviews which run the "middle ground" between those of the medical throwaways and those of the esoteric basic science or subspecialty journal are badly needed.

3. The immediate and eventual clinical significance of much basic investigation is not brought quickly enough nor adequately to the attention of the enlightened clinician who should, with minimal lag time, be prepared to use in clinical investigation and bedside practice new knowledge discovered by the basic science worker.

4. I believe that we should have an occasional outlet for hypothesis and speculation, provided the distinction between fact and fancy is made crystal clear. This would be comparable to the Lancet department called "hypothesis," which often provides fascinating and stimulating reading.

5. Priority, when urgent, can be provided by the Journal. We can, by going directly into page proof, have publication with a lead time of only a month and a half to two months.

6. The Journal is combed by all the major indices, among them Biological Abstracts, Chemical Abstracts Service, Life Sciences and Clinical Sciences, and Index Medicus. It is published six times a year, has a circulation of 2,000 and a much larger readership because of its wide library distribution.

7. Publication in The Mount Sinai Journal of Medicine is an important mechanism for making one's work known to members of the Hospital family as well as all the other components of our Medical Center.

It is demeaning that the Editor go hat in hand soliciting articles for publication. Many years ago in my early days as Editor, each chief of service, department head, or division chief voluntarily accepted a quota for the year, depending on the size of the department. This practice no longer exists. Perhaps one should consider its revival (See Exhibit A). Eight hundred to a thousand papers are written each year from our staff; a tithe should provide all the material we need. We opened our pages to extramural papers several years ago. This, too, is a source to be exploited more effectively, most particularly among Hause Staff and Residents now working elsewhere in the country.

A practice which I understand is in use at the Mayo Clinic and at Johns Hopkins is to have a first report on a significant piece of original work appear in the institutional journal, as a preliminary report, later to be elaborated into the definitive paper in a specialized journal. This device links the work to the institution from which it comes, as quotations always include the name of the journal in which the earliest report was made. When I stumbled on the now important observation that serum acid phosphatase was elevated in Gaucher's disease, I reported it first on the basis of eight cases in Volume XXIII of The Journal of The Mount Sinai Hospital. A definite study of a larger series with differential enzyme inhibitors to distinguish this from acid phosphatase originating in carcinoma of the prostate and in red cell hemolysis was later done and reported upon in the American Journal of Medicine. For anyone combing the literature in this field, Tuchman et al in the J. Mount Sinai Hosp. is more useful to our institution than Tuchman et al in the Am. J. Med. Parenthetically, over the years as many requests for reprints for the one as for the other have arrived. I do not suggest that at this time such an arrangement be made obligatory, but submit that we compete these days as much as institutions as individuals and that in time such an arrangement might naturally follow.

It is with mixed emotions that I relinquish the duties of Editor—pride in what has been accomplished, regret that more and better were not achieved, and hope that under my successor the Journal will be the unquestioned leader in its field. The New England Journal of Medicine started from a base of five hospitals in Boston, not much more than we have now.

The production of the Journal technically has always been first rate; the diligent and *devoted working Editorial Staff remains; and I would hope that the Editorial Board, drawn from our senior and prestigious leaders, will assume a more active role than it has in the past. My interest in the Journal will be no less than it has always been and I shall be at the service of my successor for any duty of his assignment. To you and the Committee on Medical Education and Publications, my sincerest thanks for twenty years of unqualified support.*

Sincerely yours,

Lester R Tuchman

Lester R. Tuchman, M.D.
Editor

cc: Dr. Thomas Chalmers
Dr. David Pomrinse
Dr. Marvin Stein

The change in title from *The Journal of The Mount Sinai Hospital* to *The Mount Sinai Journal of Medicine* implies a broadening of purpose and an increase in cultural, pedagogical, and socioeconomic responsibilities. The present Editor, with the assistance of Dr. Tuchman as Editor Emeritus and of the Editorial Staff, intends the Journal to participate in the educational program of the Medical Center, for the medical student body, the Hospital medical staff, and the postgraduate physicians. We hope the Journal will serve as a medium of exchange between basic scientist and clinician, between student and educator, betweeen Medical Center and community, and finally between alumnus and Mount Sinai.

Having worked with Dr. Tuchman for approximately twenty years of his quarter century of service to the Journal, I find it easy as his successor to express the appreciation of the Editorial Staff and Board for his wise leadership, his hard work and devotion, and for the training he has given us who must take up the responsibilities he had relinquished. We invite him to continue to actively assist the Journal as Editor Emeritus.

The appreciation of Dr. Tuchman's contribution to the Center is best expressed by a resolution passed by the Medical Board of the Hospital:

RESOLUTION

WHEREAS The Medical Board of The Mount Sinai Hospital has been advised by the Committee on Medical Education that Dr. Lester R. Tuchman has retired from the editorship of The Mount Sinai Journal of Medicine, and

WHEREAS Dr. Tuchman has rendered extraordinary service for more than two decades as a prime force in the success of this esteemed publication, and

WHEREAS we will sorely miss his scholarship and editorial judgment which contributed in such great measure to the respect which the medical profession holds for The Mount Sinai Journal of Medicine, and

WHEREAS he has been actively associated with The Mount Sinai Hospital since his internship in 1927, and

WHEREAS he has served Mount Sinai diligently throughout this period attaining his present rank as consultant physician in 1973, and

WHEREAS he was a key figure in the Third General Hospital, the Mount Sinai team during World War II, earning the rank of Lieutenant Colonel, and

WHEREAS he has been a treasured friend and adviser to physicians and patients alike

Now, THEREFORE BE IT RESOLVED that the Medical Board of Mount Sinai Hospital record herein this expression of our undying gratitude to Dr. Tuchman, inscribing his name in these annals to make known for succeeding generations the quality and scope of his contribution to the preeminence of this institution.

The Mount Sinai Hospital Medical Board

May 21, 1974

The old order changeth, making way for the new, and
God fulfills himself in many ways.

David A. Dreiling, M.D.
Editor-in-Chief

Hypertension and Anxiety. A Trial with Epinephrine and Norepinephrine Infusion

NICOLAS D. VLACHAKIS, M.D.,* DAISY DE GUIA, M.D.,* MILTON MENDLOWITZ, M.D.,† SIMONNE ANTRAM, M.D., AND ROBERT L. WOLF, M.D.

Introduction

The common occurrence of transient fluctuations of blood pressure with emotional stress is a well-documented clinical and laboratory observation. Ruskin et al (1), examining 180 survivors of the Texas City nitrate explosion, observed that 57% of the victims had diastolic blood pressures above 95 mm Hg for a period of one to two weeks after the disaster. Hickam and his coworkers (2) in a careful study of 23 normal students before and after academic examinations, found that a small number of them developed elevated blood pressures and increased peripheral resistances. Funkenstein and his associates (3) studied the cardiovascular responses to anxiety and anger. They observed increased blood pressure, stroke volume, heart rate, and cardiac output with anger and anxiety and reduced peripheral resistance with the latter. Forty-eight hypertensive and normotensive subjects were exposed to pain, fear, and anger by Schachter (4). A predominantly epinephrine-like effect was observed in the majority of the subjects during fear; and a predominantly norepinephrine-like effect was seen in most subjects experiencing pain. Either effect or a mixture of both was found during anger.

Folkow and Rubinstein (5) upon stimulation of the hypothalamic defense area in rats, induced an alerting response which was accompanied by increases in blood pressure, heart rate, and muscle blood flow. Grant and his coworkers (6) also upon hypothalamic stimulation in 14 rats found high catecholamine levels (especially epinephrine) in adrenal vein blood. Welch and Welch (7) found marked enlargement of the adrenal glands and increased adrenal medullary catecholamines in mice exposed to brief daily stress for 10 to 15 days, whereas Henry and his group (8) achieved a sustained elevation of systolic blood pressure in mice exposed to psychological stimuli for 6 to 12 months. Similar results in rhesus monkeys (9) were reported by Forsyth and Harris.

Epinephrine has been found to induce anxiety when administered to human subjects. Pollin and Goldin (10) noted increased arousal, narrowing of the

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field of attention, and subjective feelings of anxiety in normal subjects and schizophrenic patients given epinephrine intravenously; and Basowitz and his coworkers (11) reported that normal persons, during epinephrine infusion, experienced feelings similar to those felt in previous spontaneously occurring anxiety attacks.

Previous studies in this laboratory demonstrated increased digital vascular reactivity to l-norepinephrine (DVR) (12) as well as decreased apparent norepinephrine secretion rate (ANESR) (13) and decreased tritiated norepinephrine uptake (TNEU) in established essential hypertension (14). Another study suggested that a group of labile hypertensive patients existed in which these tests were negative, and self-determined home blood pressures were normal (15). It was suggested that this group of patients might be similar to those described by Frohlich et al (16) as hyperreactive to isoproterenol. It was also suggested that these labile hypertensive patients developed sporadic hypertension because of anxiety and that this condition could in some cases be superimposed on hereditary essential hypertension.

Since we were already testing patients for increased reactivity to l-norepinephrine, we decided to test their responsiveness at the same session to epinephrine as well because epinephrine is a natural substance believed to be secreted during anxiety states (17) and its intravenous infusion is relatively easy to control. In addition, in order to make comparisons and establish correlations, the patients were graded with reference to susceptibility to anxiety by a series of ordinary clinical questions. In this way, the relationship between susceptibility to stress or anxiety and vascular reactivity to epinephrine might be clarified particularly in labile but also in established essential hypertension.

Methods

Studies were carried out in 13 normal volunteers and 33 hypertensive patients, many of whom had labile fluctuations and two of whom had documented renal artery stenosis. Labile hypertension was defined by observing one or more blood pressures, well below 140/90 together with many above that level. In all subjects, medication was withdrawn for at least two weeks prior to testing. Routine testing consisted of physical examination and laboratory procedures including electrocardiogram, chest x-ray, rapid sequence intravenous pyelogram, urinary catecholamine metabolite assay, serum and urine electrolytes, thyroid function tests, urinalysis, and multichannel automated blood chemistries. Patients with coronary artery disease, cardiac arrhythmias, stroke, thyroid disorders, and azotemia were excluded. The clinical data including age, sex, and race are presented in Tables I and II.

Each subject during the first thirty minutes of his or her arrival in the laboratory was interviewed by the same investigator under similar environmental conditions. Arbitrary clinical criteria were used to evaluate susceptibility to anxiety, and four grades were established in accordance with the subject's response to six questions (Table III). After the interview, the digital vascular

TABLE I

Clinical Data in the Hypertensive Group

Grade of Susceptibility to Anxiety	Number of Cases	Mean Age (years) Range	Sex Distribution	Race	Family History Hypertension + positive − negative	Duration Hypertension (years) Mean and Range	Mean & Range Blood Pressure systolic/diastolic (mm Hg)	Mean Resting Heart Rate (beats/min)	Type of Hypertension S = sustained L = labile
I	9	47.3 20-61	6 F 3 M	6 C 3 N	6 + 3 −	4.7 1-15	143/88 110/80-188/120	80	2 S 7 L
II	6	60.3 44-70	4 F 2 M	2 C 2 N 2 PR	5 + 1 −	9.5 1-26	156/96 110/78-125/95	93	5 S 1 L
III	11	50.6 29-76	7 F 4 M	6 C 5 PR	7 + 4 −	11.4 2-20	163/92 120/80-210/110	81	7 S 4 L
IV	7	43.7 21-62	4 F 3 M	3 C 4 PR	5 + 2 −	6.7 1-15	163/104 150/95-170/120	87	5 S 2 L

F = Female; M = Male; C = Caucasian; N = Negro; PR = Puerto Rican.

TABLE II

Clinical Data in the Normotensive Group

Grade of Susceptibility to Anxiety	Number of Cases	Mean Age (years) Range	Sex Distribution	Race	Family History Hypertension + positive − negative	Mean Resting Heart Rate (beats/min)	Resting Blood Pressure systolic/diastolic (mm Hg) Mean & Range
I	6	24.2 22-27	2 F 4 M	4 C 1 N 1 PR	4 + 2 −	79.7	116/69 110/70-143/90
II	4	24.3 22-27	4 M	2 C 2 N	3 + 1 −	71	117/69 98/65-125/75
III	3	33.3 24-44	1 F 2 M	1 C 1 N 1 PR	2 + 1 −	89.3	135/82 140/70-138/85

F = Female; M = Male; C = Caucasian; N = Negro; PR = Puerto Rican.

reactivity (DVR) to epinephrine and norepinephrine was carried out in four phases. *Phase A:* Thirty minutes of complete supine relaxation with the room temperature between 26 and 29°C at which time resting cuff brachial blood pressure and heart rate were measured. *Phase B:* After vasodilatation was produced by indirect heating with an electric blanket until positive heat balance manifested by profuse perspiration was attained, a ganglion blocking agent [(trimethaphan camphorsulfonate (TMCS)] was infused at a dosage of 0.5 to 0.8 mg/min for 15 to 20 minutes, until the brachial blood pressure was stabilized at a level of 20 to 40 mm Hg below the resting blood pressure. *Phase C:* Keeping the conditions in Phase B constant, the infusion was replaced by another one containing epinephrine chloride in addition to TMCS and was given for 15 to

TABLE III

Arbitrary Clinical Criteria for Grading of Susceptibility to Anxiety

Criteria:

History in stressful situations of:

1) Sweating
2) Flushing
3) Voice changes or excessive swallowing
4) Feeling of rapid heart action
5) Muscular tension or feeling of internal shakiness
6) Tremor or hyperventilation

Grading:

I. No susceptibility to anxiety if none of above criteria are present
II. Mild susceptibility to anxiety if one or two of above criteria are present
III. Moderate susceptibility to anxiety if three or four of above criteria are present
IV. Severe susceptibility if five or all six of above criteria are present

17 minutes at a flow rate of 0.1 μg/Kg/min. *Phase D:* Keeping the conditions in Phase B constant and 30 minutes after the end of Phase C, an infusion of levarterenol bitartrate (3 mg levarterenol in 250 ml 5% dextrose solution) in addition to TMCS was given for 15 to 17 minutes. The intravenous flow rate was kept the same as in previous phases unless an excessive response of the blood pressure necessitated a lower flow rate. This was the only phase in which flow rate was occasionally altered.

In all four phases, the digital blood flows (calorimetric) and digital blood pressures were measured with a Gaertner capsule using the flush-throb method. Mean blood pressure was determined by adding one-third of the pulse pressure to the diastolic pressure. Effective mean pressure was measured by subtracting a calculated venous pressure and critical closing pressure from the mean pressure. The latter was needed only for phases C and D. Radius equivalents were then determined from Poiseuille's law using a length constant. The work of vasoconstriction based upon the epinephrine and norepinephrine infusion rate was then calculated and is expressed in ergs per μg of infused epinephrine (E) or NE per minute. The following formula was used (12):

$$w = 197\, p_1(r_1^2 - r_2^2) - \frac{16.1\, p_1^2(r_1^6 - r_2^6)}{Q_1}$$

in which w is work in ergs, p_1 is effective mean pressure, and Q_1 is blood flow in the dilated state (Phase B); r_1 is radius equivalent during vasodilatation (Phase B); r_2 is radius equivalent in the resting state (Phase A); and r_3 and r_4 are the radius equivalents during Phases C and D, respectively, replacing r_2 in the formula when the work of vasoconstriction produced by E or NE is measured. In addition, brachial cuff blood pressures and heart rates were recorded just before and at the end of each phase. The entire procedure lasted approximately two hours. During the procedure no subjective effects were experienced by the subjects during NE infusion. During the E infusion, however,

TABLE IV

Grading of Ability to Tolerate the Procedure According to Clinical Manifestations during Epinephrine Infusion

Manifestations:

1. Discomfort
2. Feeling of rapid heart action
3. Feeling of internal shakiness or muscular tension
4. Fear
5. Tremor
6. Hyperventilation or extreme fear

Grading:

I. Good if only one or two of the first three manifestations are present
II. Fairly good if only three of the first five manifestations are present
III. Poor if either extreme fear or hyperventilation is experienced

subjective symptoms were experienced and these were scored and graded by criteria outlined in Table IV.

On a subsequent day, in all the normotensive and in 22 of the hypertensive patients, the apparent norepinephrine secretion rate (ANESR) was measured as follows: 6.2 μg (500 μCi) of tritiated NE was injected intravenously over a period of three minutes, following which a 24-hour urine was collected. The specific activity of normetanephrine (NM) was then measured in an aliquot. The ANESR represented the counts of tritium given divided by the specific activity of NM.

Results

The data in the hypertensive group are presented in Tables V and VI. There was no significant change from grade to grade of susceptibility to anxiety in the mean age, sex or race distribution or the duration or type of hypertension whether labile or sustained. Although the mean resting blood pressures were somewhat higher in grade IV, the differences between grades was not statistically significant. There was also no significant relationship between the grade of susceptibility to anxiety and the mean resting heart rate.

On the other hand, the DVR to E increased significantly from grade to grade. In grade I patients, the DVR to E ranged between −83 and +80 with a mean of +30. In grade II, this value was between +103 and +217 with a mean of +156. This differed significantly from grade I ($p < 0.01$) and grade III (mean +274; $p < 0.05$). In grade IV, the DVR to E ranged from +168 to +694 with a mean of +426. The difference from grade III, however, was not statistically significant. On the other hand, the difference between grades III and IV grouped together and grades I and II as a group (Table VI) was highly significant ($p < 0.001$). The DVR to NE was normal in 19 hypertensive subjects and above normal in 14 hypertensive subjects but was not significantly related to the grade of susceptibility to anxiety. It was, however, weakly correlated with DVR to E ($r = 0.36$) ($p < 0.05$). While the correlation in the hypertensive group between the extent of the abnormality in the DVR to NE and the extent of the

TABLE V

Hypertensive Group

	Grade of Susceptibility to Anxiety	Digital Vascular Reactivity to		Apparent Norepinephrine Secretion Rate	Circulatory Changes during E Infusion		
		E	NE		Heart Rate (beats/min)	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)
Range	I	−83 to +80	7N, 2A	5N, 2A	+2 to +24	−10 to +60	−23 to 0
Mean		+30	157		+12	+11	−10
S.E.M.		17			2.4	6.9	2.6
Range	II	+103 to +217	3N, 3A	3N	+6 to +28	−3 to +28	−22 to −5
Mean		+156	211		+19	+13	−14
S.E.M.		21			3.9	4.6	2.8
p		<0.01	ns		ns	ns	ns
Range	III	+138 to 554	4N, 7A	3N, 4A	+2 to +32	0 to +52	−27 to +8
Mean		+274	267		+18	+28	−11
S.E.M.		41			3	4.2	3.6
p		<0.05	ns		ns	<0.05	ns
Range	IV	+168 to +694	5N, 2A	2N, 3A	+16 to +52	+2 to +60	−40 to +9
Mean		+426	249		+31	+31	−7.7
S.E.M.		64			4.5	7.9	6.8
p		ns	ns		<0.05	ns	ns

E = epinephrine; NE = norepinephrine; ns = not significant; p = probability; B.P. = blood pressure; N = normal; A = abnormal; S.E.M. = standard error of the mean.

Significance testing was done between successive grades, using the Student's "t" test.

TABLE VI

Hypertensive Group

	Group of Susceptibility to Anxiety	Number of Cases	Digital Vascular Reactivity to E	Circulatory Changes during E Infusion		
				Heart Rate (beats/min)	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)
Mean	A	15	+80	+15	+12	−12
S.E.M.			21	2.2	4.4	1.9
Mean	B	18	+333	+23	+29	−9.8
S.E.M.			39	2.9	3.9	3.3
p			<0.001	<0.05	<0.025	ns

Group A comprises grades I and II of susceptibility to anxiety.

Group B comprises grades III and IV of susceptibility to anxiety.

Symbols as in previous tables.

abnormality in the apparent NE secretion rate was weak ($r = 0.4$) ($p < 0.1$) in only four cases and two additional borderline cases out of the 33 hypertensive patients was there a discrepancy between the two tests with reference to whether they were within the normal range or definitely abnormal. The discrepancies were always in the same direction (abnormal DVR and normal ANESR) and were probably attributable, at least in part, to incomplete urine collection (Table V).

In all 13 normotensive subjects, the DVR to NE and the ANESR were normal (Tables VII and VIII). The same criteria for scoring susceptibility to anxiety yielded six cases in grade I, four in grade II, and three in grade III. None of the normal volunteers tested could be classified as grade IV. In this normotensive group, the DVR to E differed significantly only between the grades II and III ($p < 0.05$) but between grades I and II grouped together compared with grade III (Table VIII), the difference was highly significant ($p < 0.005$), despite the small number of cases in grade III. When hypertensive and normotensive subjects were compared grade for grade, DVR to E was not

TABLE VII

Normotensive Group

	Grade of Susceptibility to Anxiety	Digital Vascular Reactivity to		Apparent Norepinephrine Secretion Rate	Circulatory Changes during E Infusion		
		E	NE		Heart Rate (beats/min)	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)
Range	I	+8 to +86	6N	6N	+8 to +36	+10 to +25	−40 to −8
Mean		+35			+19	+18	−19
S.E.M.		13			4.2	2.3	4.5
Range	II	+49 to +121	4N	4N	+4 to +32	+18 to +35	−27 to −3
Mean		+76			+14	+30	−17
S.E.M.		20			6.6	4.1	5.2
p		ns			ns	ns	ns
Range	III	+149 to +241	3N	3N	+4 to +16	+7 to +15	−48 to +5
Mean		+201			+9.3	+12	−20
S.E.M.		27			3.5	2.4	15
p		<0.05			ns	<0.05	ns

Symbols as in Table V and in previous Tables.

TABLE VIII

Normotensive Group

	Group of Susceptibility	Number of Cases	Digital Vascular Reactivity to E	Circulatory Changes during E Infusion		
				Heart Rate (beats/min)	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)
Mean	A	10	+51	+17	+23	−18
S.E.M.			13	3.5	2.8	3.2
Mean	B	3	+201	+9.3	+12	−20
S.E.M.			27	3.5	2.4	15
p			<0.005	ns	<0.025	ns

Group A comprises grades I and II of susceptibility to anxiety.
Group B comprises grades III and IV of susceptibility to anxiety.
Symbols as in Table V and in previous tables.

TABLE IX

Significance Levels of Epinephrine (E) Reactivity between All Hypertensive and All Normotensive Subjects

(in ergs per μg E per minute)

	Mean	S.E.M.	p
Normotensive (13) versus Hypertensive (31)*	85 vs 218	22 vs 31	<0.005
Normotensive (13) versus Hypertensive with abnormal Digital Vascular Reactivity to NE (13)	85 vs 196	22 vs 46	<0.02
Normotensive (13) versus Hypertensive with normal Digital Vascular Reactivity to NE (18)	85 vs 226	22 vs 43	<0.05

* *Note:* The renovascular patients were excluded.
Symbols as in previous tables.

significantly different except in grade II where the mean in the hypertensive group was somewhat greater ($p < 0.05$). There was no significant correlation between DVR to E and to NE in the normotensive group. The correlation between DVR to NE and ANESR in the normotensive group was $r = 0.63$ ($p < 0.05$). In both normotensive and hypertensive groups together it was $r = 0.47$ ($p < 0.01$).

When the hypertensives as a whole were compared with the normotensives as a whole (Table IX), reactivity to E was greater in the hypertensive group ($p < 0.005$) as one would expect from the increased incidence of grade III and IV anxiety in the hypertensive group. Also, when the hypertensive group was divided between those with high DVR to NE and those with normal DVR to NE and compared with the normotensive group, reactivity to E was significantly greater in each hypertensive group ($p < 0.025$ and < 0.05 respectively) than in the normotensive group. On the other hand, no significant difference in reactivity to E could be demonstrated between the two hypertensive groups namely those with normal responsiveness to NE and those with increased NE responsiveness.

The heart rate changes during E infusion in the hypertensive group had a tendency to increase with increased grades of susceptibility to anxiety but this was significant statistically only between grades III and IV ($p < 0.05$; Table V). In the normotensive group, however, the heart rate changes during E infusion had a tendency to decrease but this was not statistically significant (Table VII). The initial resting heart rates were variable and could not be related significantly to the grading. The changes in brachial blood pressure during E infusion were not statistically significant between grades except for a modest increase in systolic blood pressure between grades II and III in the hypertensive group only ($p < 0.05$) (Table VII).

Using the criteria in Table IV, all but two subjects tolerated the procedure well. In the two exceptions, the E infusion was discontinued after 10 minutes because of uncontrolled fear and hyperventilation. Both these subjects were hypertensive and were graded IV in susceptibility to anxiety with DVRs to E of 527 and 694.

Discussion

The results in our studies with reference to changes of heart rate during the infusion of E were not well correlated with the grading of susceptibility to anxiety. In the hypertensive group the diastolic blood pressure decreased less in the more anxious subjects and this was opposite to the changes in diastolic blood pressure in the normotensive group. Cardon and his coworkers (19) found higher heart rates in schizophrenic patients (where anxiety is a prominent feature) than in normal controls, although the increase in heart rate during E infusion was significantly less in the schizophrenic patients than in normal subjects. Similar results were also observed by Freeman and Carmichael (20) who also found a smaller increase in systolic and a lesser decrease in the diastolic during E infusion in schizophrenic patients as against normal subjects. It is possible that these schizophrenic as well as our hypertensive patients react to anxiety or stress by increasing peripheral resistance whereas normal subjects react to such stress by increasing cardiac output. The number of cases in each hypertensive and normotensive group, however, is insufficient for a firm conclusion in this respect.

It seems reasonable to conclude from the data that DVR to E is best related to the grade of susceptibility to anxiety in both the normotensive and hypertensive groups. Also, this susceptibility to anxiety may or may not be associated with hypertension. In the labile hypertensive group it may be the only factor responsible for the observed hypertension. This is more apparent in patients with higher grades of susceptibility to anxiety but could not be clearly demonstrated in those with lower grades. In the essential hypertensive group as defined by increased DVR to NE and decreased ANESR, susceptibility to anxiety and increased DVR to E may be factors increasing the basic hypertension. The validity of defining essential hypertension by the tests indicated is supported by finding them positive in a large series of cases of established long-standing relatively fixed essential hypertension (13, 22).

E is both a beta and alpha receptor stimulator (18). In small doses it is largely a beta receptor activator and in larger doses both a beta and alpha receptor stimulator. Duff (21) demonstrated increased vasoconstriction in the hand when E was administered intraarterially in hypertensive subjects. No effort was made by him to define the anxiety factor or to stabilize the circulation by indirect heating or by ganglion blockade prior to injection. It is impossible to determine from our data whether the vasoconstriction observed was a direct effect of E infusion or attributable to breakthrough of the blockade caused by generalized beta receptor stimulation by E, thus orchestrating a combined alpha and beta stimulation. Vasodilatation in the digit in response to E infusion was observed only in a few subjects with little or no susceptibility to anxiety who are classified as grade I. The test with E seemed better tolerated than the isoproterenol tests (16) and relatively safe in the labile hypertensive subject.

It is of interest to note that beta receptor blocking drugs such as propanolol

(23) and practolol (24, 25) are often effective in patients with labile hypertension as well as in those with anxiety itself. The mechanism of this effect is uncertain but it seems that anxiety as well as anxiety hypertension may be attributable as much to increased reactivity to circulating E as to increased release of E into the circulation.

Summary

Epinephrine (E) as well as l-norepinephrine (NE) was infused during ganglion blockade in 33 hypertensive and 13 normotensive subjects and circulatory responses including digital vascular reactivity (DVR) were recorded. The procedure was well tolerated in all but two subjects. On a subsequent day the apparent norepinephrine secretion rate (ANESR) was measured in every normal subject and in 22 of the hypertensive patients. The hypertensive subjects included patients with established essential hypertension, patients with labile hypertension presumably attributable at least in part to anxiety and two patients with renovascular hypertension. Four grades of susceptibility to anxiety were established in these subjects using arbitrary clinical criteria. The relation between the grade of susceptibility to anxiety and heart rate and brachial blood pressure response to E or NE infusion was largely insignificant. On the other hand, the relation between the digital vascular response to E infusion and the grade of susceptibility to anxiety was highly significant in contrast to the lack of significant relationship between DVR to NE and the grading. There was significant correlation of low degree between DVR to E and DVR to NE in the hypertensive group only. In severe grades of susceptibility to anxiety, increased reactivity to E seemed to be the major factor producing the hypertension. In fact, anxiety and anxiety hypertension may be attributable as much to increased responsiveness to E as to excessive secretion of E. In all but a few cases the ANESR was normal or abnormal when the DVR to NE was normal or abnormal respectively. The extent of the abnormality, however, was significantly but only moderately well correlated between the two tests.

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Early Twentieth Century Origins of Modern Blood Transfusion Therapy

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Modern use of blood and blood components is an outgrowth of events that occurred during the first half of the twentieth century. Previously, human blood had been used therapeutically for at least several hundred years but the procedure had been empiric and usually of dubious value. Reliable transfusions evolved from the development of test procedures and methods essential for their safety, and this report will attempt to describe the discovery of these measures.

Much of the development of transfusion therapy occurred in New York City and at The Mount Sinai Hospital. Having been at this institution for 27 years, the author has long known about some of the events that took place and has had an opportunity to know personally many individuals who were directly responsible for progress in this subject. Nevertheless, considerable indulgence is necessary because, despite extensive search of available literature and lengthy conversations with active participants, the task is not an easy one. However, if one looks at the overall picture, and even beyond the many contributions of staff members of The Mount Sinai Hospital, a provocative story does indeed emerge.

A widespread misconception concerning biomedical progress is to assume its strict dependence on basic discoveries and unusually perceptive minds. This assumption is often untrue, and the development of modern transfusion therapy is a clear example of how incorrect it can be.

Modern transfusion therapy did not begin precisely with Karl Landsteiner's description of types O, A, and B in 1900 (1) and 1901 (2). In fact, there is every reason to think that very few people paid much attention to the scientific reports for which Landsteiner was later to receive the Nobel Prize in Medicine (3). But this was only one of several important reports to be ignored. Another was made in 1892 by Ziemssen. This prominent Munich internist described an extraordinarily simple method for conducting a direct transfusion with multiple syringes, a needle in an antecubital vein of the donor and another needle in an antecubital vein of the patient (4). Each needle was cuffed with rubber tubing so that it could be clamped off when not connected to a syringe, and it is likely that very little blood was spilled in the course of a transfusion. But no one paid attention to this report either, perhaps because Ziemssen was already on record for espousing subcutaneous blood transfusion (5). His multiple syringe method was independently rediscovered 21 years later by Dr. Edward Lindeman (6), then an intern at Bellevue Hospital in New York City.

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Lindeman became a famous transfusionist before he completed his internship. Lindeman's method, however, was not as efficient as Ziemssen's: Lindeman used cannulas rather than needles, and these often became occluded by clotted blood.

Modern interest in blood transfusion was actually stimulated by Alexis Carrel and his description of end-to-end blood vessel anastomosis in 1902 (7). Carrel and his work influenced George Crile (8), the great Cleveland thyroid surgeon, who published a cannula method for direct transfusion in 1907 (9), and a text, "Hemorrhage and Blood Transfusion," in 1909 (8). Crile gave hundreds of surgical blood transfusions, most of them successful, but he paid little or no attention to ABO types. Crile knew about Landsteiner because Ludwig Hektoen, the Chicago pathologist, told him about this work (8). But Crile was more impressed by the monumental studies of Ehrlich and Morgenroth (10–12), and thought that a good test for hemolysis would surely provide ample protection. Crile's tests were quite elaborate (8), but they did not always prevent disastrous reactions, especially in debilitated patients (8).

While Hektoen had little influence on Crile, Richard Weil, then Pathologist at German Hospital (now Lenox Hill Hospital) in New York, alerted a senior intern, Reuben Ottenberg (3) to the importance of Landsteiner's blood types. Independently of Crile, Ottenberg had become interested in the possibilities of transfusion therapy through a cannula modification of the surgical approach of Alexis Carrel (3), and Ottenberg actually performed cannulated direct blood transfusions in 1907 (13) without knowing about Crile's work (3). In 1908, after he was denied a position at Rockefeller Institute (3), Ottenberg came to The Mount Sinai Hospital where he found the surgeons, Charles Elsberg (14) and Leo Buerger, to be interested in transfusion therapy. Until 1913, the method used at The Mount Sinai Hospital was anastomosis by cannula, but even when both the donor and patient in this procedure were monitored for weight change to determine how much blood was being given (15), thrombosis all too often prevented an adequate transfusion.

Ottenberg probably performed New York City's first blood transfusions, and he did so using both Landsteiner's ABO typing for patient and donors, and modifications to further ensure serologic compatibility (3). Credit for the first transfusion has been given mistakenly to Carrel (16) for a very dramatic and life-saving transfusion of a newborn with melena neonatorium, Vitamin K deficiency. The patient was the daughter and first child, of Dr. Adrian V. S. Lambert, clinical professor of surgery at Columbia's College of Physicians and Surgeons. The father's brothers were also physicians, Dr. Alexander Lambert, president of the American Medical Association in 1919, and Dr. Samuel Lambert, Dean of The College of Physicians and Surgeons. Carrel had arrived at Rockefeller Institute in 1906, by way of the University of Chicago (1904), from Lyon, France. Apparently, the Lambert brothers had heard nothing of Ottenberg and begged Carrel to perform a transfusion even though Carrel had had no previous human experience. The transfusion was brilliantly conducted (16) but, unlike Ottenberg's earlier transfusions, was performed without benefit of blood typing or testing for compatibility (16). So, perhaps the success of this transfusion was largely fortuitous despite Carrel's skill.

Ottenberg contributed much to successful transfusion therapy and was responsible for a number of important concepts. He was the first to use both ABO typing and compatibility testing prior to transfusion (13, 17), and he was the first to suggest inheritance of ABO types (17). He associated lysis in vitro with posttransfusion hemoglobinuria (18), and agglutination without lysis in vitro both with jaundice and erythrophagocytosis in the patient's posttransfusion blood samples (18, 19). He was the first to explain the relatively minor role of donor antibodies and, thus, the universality of type O donors (19). Another major concept, and one still not fully appreciated, was his observation that lysis in vivo was much more marked than lysis in vitro (20). Although Ottenberg met considerable resistance in performing tests prior to transfusion (3), there were many interested people who paid attention to his work. Furthermore, the remarkably consistent progress in transfusion therapy which occurred at The Mount Sinai Hospital from 1908 to 1916, was closely associated with great rivalry between staff members at this hospital. Perhaps it was actually these very intense personal differences which assured a supply of interested parties to exploit every emerging idea.

In contrast to Ottenberg, we have the strange fate of the serologist, Carlo Moreschi, a brilliant Italian Jew working in Germany. In 1908, Moreschi described the antiglobulin test (21) but, although his report was duly noted by Landsteiner (22), no one paid the slightest attention. The entire principle had to be rediscovered in 1945 by Coombs, Mourant, and Race (23). Moreschi also contributed much to complement fixation tests, but died prematurely of influenza in 1917.

In a brief three-month period at the end of 1914, four independent investigators described the use of sodium citrate as an anticoagulant for human donor blood. The first of these scientific reports came in November 1914 from Albert Hustin (24), an experimental surgeon who used an anticoagulant solution containing salt, citrate, and glucose. Hustin's pioneering endeavors were later recognized by a commemorative bronze plaque conspicuously displayed at his school, The Free University of Brussels, and by naming the Albert Hustin Blood Transfusion Centre in his honor.

The other three reports all appeared in January 1915, and they seem to have been precipitated by an American newspaper release from Luis Agote, Buenos Aires, Argentina, in November of 1914 (25). Agote described the correct dose of sodium citrate, work probably performed by his associate, Imaz Apphatie, and a pressure method for administration of citrated blood (26, 27). Agote was ultimately responsible for numerous safe blood transfusions but later confessed that he paid little heed to human ABO types (28). Agote must have used blood donors of South American Indian parentage; they were readily available, and all are type O!

The most important scientific reports on citrate were made by two New

Yorkers, Richard Lewisohn, a surgeon at The Mount Sinai Hospital, and Richard Weil. Weil, on the visiting staff of Mount Sinai, had been the Pathologist at German Hospital who alerted Reuben Ottenberg to Landsteiner's ABO types. In 1915, Weil was Assistant Director of Research at The General Memorial Hospital (now The Memorial Hospital Center for Cancer and Allied Diseases); he became Director of Research later that year but, like Carlo Moreschi, died of influenzal pneumonia in 1917 (29). Both Lewisohn and Weil contributed much to the citrate story, but they did so in different ways.

Lewisohn was interested in anticoagulating donor blood in 1911, when he came to George Baehr for advice. At Baehr's suggestion and under Baehr's guidance (30), Lewisohn began systematic studies of citrate toxicity in dogs. Lewisohn, unduly fearful of citrate toxicity, carefully developed the minimal amount of citrate that would anticoagulate human blood for several hours (0.2% final concentration), and he proved that this would be completely safe even if 2,500 ml of donor blood were given in a brief period of massive transfusion (31). Unfortunately, Lewisohn was unaware of the 1911 report of Holt and Penfold (32) that some batches of stale distilled water caused pyrexial reactions. In consequence, Lewisohn was later needlessly obligated to defend his citrate toxicity data before an onslaught of attacks and innuendos leveled by transfusionists who used direct methods. Lewisohn's citrate transfusions were associated with a fifty percent incidence of chills and fever whereas, for example, Unger's direct transfusions were associated with only ten percent reactions (33). Only after Seibert's 1923 report of bacteria causing pyrogen reactions (34) did the truth emerge. Finally, Lewisohn and Nathan Rosenthal in 1933 (35) described the then new Central Supply System of The Mount Sinai Hospital as the most feasible means for eliminating pyrogen reactions both from blood transfusion and intravenous solution therapy.

Despite Lewisohn's considerable contributions to citrated blood therapy, no one has seen fit to honor him with either a plaque or a named transfusion center. The Blood Bank at The Mount Sinai Hospital was named after Irving Geist, a successful business man who donated $250,000 to the hospital. Strangely, there is only one source of pyrogen-tested sodium citrate solution available today in the U.S.A., and this is essentially Lewisohn's formulation, 50 ml of 2.5% made by Eli Lilly. Another solution was available until a year ago; this was 1.17 grams in 5 ml, approximately Agote's formulation.

Richard Weil's contributions were quite different. He made no attempt to determine optimal citrate dosage but merely used the same amount of citrate that had evolved empirically to keep laboratory blood specimens fluid (1% final concentrations). Weil was not interested in massive blood transfusions and used only 300 ml donations rather than Lewisohn's 500 ml. But Weil noted two phenomena in his January 1915 report (36): citrated blood could be stored in a refrigerator for at least several days before use (the first suggestion for blood banking), and serological tests for compatibility between patient and donor could be performed on whole citrated blood samples if one made mixtures in the ratio of 9:1 and 1:9. The latter suggestion was, in fact, a forerunner of the slide test for detection of Rh antibodies developed in 1945 by Diamond and Abelson (37). Both suggestions caught the eye of Peyton Rous at Rockefeller Institute.

Late in 1915 (38) Rous and Turner described an improvement of Weil's compatibility test in which they used drop quantities of blood from the ear lobe (citrated, of course, but by pipette) to construct whole blood mixtures between recipient and donor in the ratios of 9:1, 1:9, and 5:5. In 1916, at the request of the U.S. Army to develop a more stable donor blood in the event that the U.S.A. would become involved in World War I, Rous and Turner (39) developed a solution to maintain the viability of refrigerated donor red cells for at least 14 days. This established blood bank feasibility, and the Rous-Turner solution remained the red cell preservative of choice until development of acid-citrate-dextrose (A.C.D. solution) in 1943 by Loutit and Mollison (40).

The Rous-Turner solution of salt, citrate, and glucose was a rather massive amount of diluent: 300 ml of donor blood required 700 ml of solution. Rous and Turner anticipated criticism on this basis and suggested (39) that the preserved blood be allowed to settle so that the supernatant fluid could be discarded. Rous and Turner recognized that the recipient needed only red cells, and they made the first proposal for packed red cell transfusions.

While the citrate story emerged, Lester J. Unger was an intern at The Mount Sinai Hospital with unusual personal problems: he was unable to get along with Mount Sinai's brilliant Chief of Medicine, Emanuel Libman. The problem may have begun just before Unger's graduation from Columbia University's College of Physicians and Surgeons. Unger became desperately ill with fever, pharyngitis, and massive lymphadenopathy, and several consultants, including Libman, made the diagnosis of acute leukemia. Infectious mononucleosis was not as yet recognized. Unger, of course, recovered, began his internship six months late, and denies (33) deriding the sensitive Libman over the mistaken diagnosis. Unger was, naturally enough after his illness, keenly interested in blood problems, including transfusion. Watching Edward Lindeman at work, and often observing more blood on the floor than reached the patient, he conceived the need for a four-way stop-cock for direct transfusion (41), and he had one made. But Libman would not permit him any patients, and Ottenberg, who was closely associated with Libman, would not teach him how to type blood or test for compatibility. So Unger turned to Peyton Rous at Rockefeller Institute to learn blood typing and the Rous-Turner compatibility tests. Unger then threatened to go to Columbia for transfusion patients if Libman continued to refuse consent at Sinai, and he won this argument. Unger began successful stop-cock transfusions in 1915 at Mount Sinai (41, 42). In 1916, Unger and Lindeman presented their respective methods at the Annual Meeting of the American Medical Association in Atlantic City, and Libman, when asked to give his opinion about the respective merits of the two approaches, sided completely with Unger. Lindeman, dismayed by this, went swimming in the surf to cool off, but suffered a cramp and drowned (33). Unger, however, began a long and successful transfusionist career, and continued to use the methods of testing he had learned from Peyton Rous. In 1921, Unger (43) described positive tests for incompatibility associated with severe clinical transfusion reactions but independent of the ABO types. These were very likely Rh antibodies, but 19 years before the report of Wiener and Peters (44). In 1923, Unger was invited to join Landsteiner at Rockefeller Institute, but accepted a simultaneous offer from the pediatrician, Alfred F. Hess (33). A year later, Landsteiner hired Philip Levine as his assistant and initiated another brilliant scientific career. It is of interest to note that Unger would have returned to Mount Sinai Hospital had Hess been made Chief of Pediatrics, but this did not happen because of adverse publicity about experimental avitaminosis in children. The dilemma over Hess and the Department of Pediatrics at Mount Sinai was resolved by inviting Bela Schick, from Austria, to become Chief of this department (30).

The period, 1939 to 1947, proved to be just as intensely productive as the period of 1908 to 1916. It began quietly with a report from Levine and Stetson (45) relating a severe case of erythroblastosis to a hitherto undescribed intra-ABO agglutinin in the mother's serum. This serum agglutinated 80% of unselected blood samples, including the blood of the father (45). In 1940, Landsteiner and Wiener, using rabbit anti-rhesus red cell serum, described human Rh polymorphism (46), and Levine was able to learn that the maternal serum he had described in 1939 displayed similar agglutinating activity. This led to studies of known instances of erythroblastosis, most of which were provided by Peter Vogel of The Mount Sinai Hospital, and publication of the very important observation that, in over 90% of instances, the mother was Rh-negative and the father Rh-positive (47). This report directly generated a broad interest in immunohematology, immunogenetics, and immunoanthropology. Not only were the Rous-Turner slide tests and the Moreschi antiglobulin tests rediscovered, but two new tests emerged. These were the use of albumin (48) and protease treatment of red cells (49) for the detection of Rh antibodies.

The whole story of the evolution of transfusion technique had, strangely, to be repeated again in its entirety for the development of exchange transfusion therapy in management of Rh disease of the newborn. Wiener and Wexler (50) developed radial artery cut-down coupled with cannulation of the saphenous vein, essentially the Crile method. Wallerstein (51) developed use of a needle in the sagittal sinus and another in the antecubital vein, essentially the Ziemssen-Lindeman method. And finally Diamond (52), using the umbilical vein approach, developed tandem three-way stop-cocks to make a four-way stop-cock, the Unger method.

Strangely, the use of Rh-negative blood for the treatment of Rh erythroblastosis was never published. A draft was completed in 1942 by Peter Vogel, but personal differences between his two co-authors, Philip Levine and Eugene Katzin, blocked submittal (53). Exchange transfusion therapy with Rh-negative blood ensued anyhow, and this was because the concept of exchange transfusions was not new. The Canadian, Bruce Robertson, in 1916, successfully treated two soldiers with carbon monoxide poisoning in France by partially exchanging their blood (54). In consequence, the Hospital for Sick Children of Toronto adopted the procedure in 1921 for the treatment of burns, erysipelas, scarlet fever, "intestinal intoxication," and sundry other conditions (54). These Canadian pediatricians used the sagittal sinus and the femoral vein, the former suggested in 1915 by Helmholz of Mayo Clinic. In fact, Hart (55) actually used these vessels for a successful 300 ml exchange transfusion of "icterus gravis" in 1925.

The major wars of this country contributed only modestly to transfusion therapy. The first World War was responsible for development of the Rous-Turner solution (39). Similarly, British research during World War II culminated in the A.C.D. solution of Loutit and Mollison (40). In addition, many physicians in military service during World War II, including this author, learned by first-hand experience how to use blood and plasma to overcome shock. The Korean conflict brought added experience with type O donor blood, while in Vietnam we increased our experience with reconstituted frozen red cells.

But scientific discovery had considerably less effect on progress. Many extraordinarily innovative ideas went unnoticed. Ziemssen's multiple syringes, Moreschi's antiglobulin test, the slide test of Rous and Turner (after Weil), and the etiology of pyrogenic reactions were ignored for years, or observed by only a tiny minority of workers. But this is not unusual in science: Mendel's laws of inheritance were ignored for 34 years and had to be rediscovered (56). In other words, a great discovery needs an appropriate audience or it will not be exploited. And, if the discovery is not exploited, it may be forgotten or drop into obscurity.

Richard Lewisohn in 1955 (57) wrote a short review of transfusion's development. In it he gave a table of blood use at The Mount Sinai Hospital, and the data for the first four points are unusually interesting. In the years, 1923, 1932, 1935, and 1941, there were respectively 143, 477, 794, and 2,097 patients transfused. These data fit best an exponential increase in blood transfusions with doubling every 5 years. On a least squares fitted line, there should have been 20 transfusions in 1910, a figure actually given in text by Lewisohn. A value of 42 in 1915 is realistic but perhaps a little low for that particularly active year. Unger (33) thought that as many as 100 may have given in 1915. An exponential growth curve implies an unlimited number of patients needing transfusion, and willing donors, with number of actual transfusions depending only on capacity to perform them. If so, World War I had little if any influence, but World War II did, but in a peculiar manner. The supply of donors would surely have decreased after 1935 had not Mount Sinai's Blood Bank been started in 1938, and even this would have been insufficient by 1941 without an inexhaustible supply of packed red cells derived from an active "Plasma for Britain" program. Sterile Karo corn syrup was added to these cells so that they could be stored at 4°C for 10 days. Later, of course, the supply of donor blood dwindled so that exponential growth of transfusions could no longer be supported. But it was not until long after World War II that we became aware of the blood-transmitted hepatitis problem and actually diminished our medical requests for blood transfusions.

It would be very satisfying to conclude this review with a statement that modern transfusion therapy no longer suffers from problems like those which existed during the first half of the twentieth century. Unfortunately, this is not the case. Scientific progress in the last half of this century appears to be no more rapid, nor any more consistent, than that in the first half. Indeed, it may be slower and less consistent. Blood typing and tests for donor blood compatibility are essential to blood banking, but not a single new routine method has been introduced since 1947; that was the year in which protease treatment of red cells was described (49). No other ancillary hospital laboratory has such a record. New blood typing tests have been found, of course, but they have not been routinely adopted as yet. The same blood banking laboratories quickly adopt every new test for the virus of hepatitis, and every new method to make better blood components, so they do not lack initiative. Apparently, however, they do not fully understand either the purpose or the mechanism of blood typing and, accordingly, they do not readily become interested in new approaches to the blood typing problem. In consequence, the compatibility of donor blood continues to carry a small risk of incorrectness. Perhaps this is not too bad as long as everyone knows about it. Certainly, the situation is far better than for tissue typing, or testing for the compatibility of either white cells or blood platelets, where we know next to nothing. Hopefully, current basic research into the nature of cell membrane protein particles will change our intellectual perspectives in regard to human blood types, and this subject will at last mature into a realistically scientific discipline.

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Some Recent Advances in Virilizing and Feminizing Syndromes and Hirsutism*

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The adrenal cortex and gonads arise in common on the urogenital ridge. It is, therefore, not surprising that they have common biosynthetic pathways to androgen and estrogen (1) (Fig. 1). The adrenal cortex, in addition, has pathways to the formation of aldosterone and to cortisol. The gonads for all practical purposes are devoid of these latter two pathways. However, at times, evidence for an ability to 11β or 21 hydroxylate has been found in the gonad, for example, in interstitial cell tumors. Such capabilities suggest either that the tissue is adrenocortical in origin rather than testicular (or ovarian) or that under certain circumstances, a repressed ability to 11β or 21 hydroxylate is resurgent by analogy to the ectopic syndromes in which polypeptide hormones are produced by a bronchogenic carcinoma. Leaving these latter considerations aside, it is easy to foresee that excessive production of androgen or estrogen may occur in the adrenal cortex or gonads or in both organs simultaneously.

At the present time, it is known that the gonads are under regulation by the gonadotropins (LH and FSH) and that the adrenal cortex is controlled by corticotropin. This latter hormone, however, has stimulatory capacities that must be stressed in order to appreciate the separation of some adrenogenital syndromes (i.e., congenital adrenocortical hyperplasia) from the virilizing and feminizing syndromes of the gonads. If one looks at the biosynthetic pathway and one assumes there to be some congenital defect facilitating the excessive production of androgen or estrogen then such a congenital defect could very likely affect the pathway both in the gonad as well as in the adrenal cortex. Similarly if there were a defect in the formation of androgen or estrogen because of an enzymic defect in the androgen-estrogen pathway, i.e., in 17-hydroxylase (affecting cortisol as well as androgen and estrogen) or 3β-ol dehydrogenase (affecting cortisol, aldosterone as well as androgen-estrogen), the congenital disorder would also be expected to be present in both the gonad and the adrenal cortex. On the other hand, the regulation of androgen and estrogen production in the adrenal cortex has an additional mechanism (2).

Corticotropin has a dual action. 1) It facilitates the production of precursor (pregnenclone) from cholesterol and it increases the cells of the zona fasciculata, the site of formation of cortisol. Accordingly, with the administration or secretion of ACTH, excessive amounts of pregnenelone are formed. These pour

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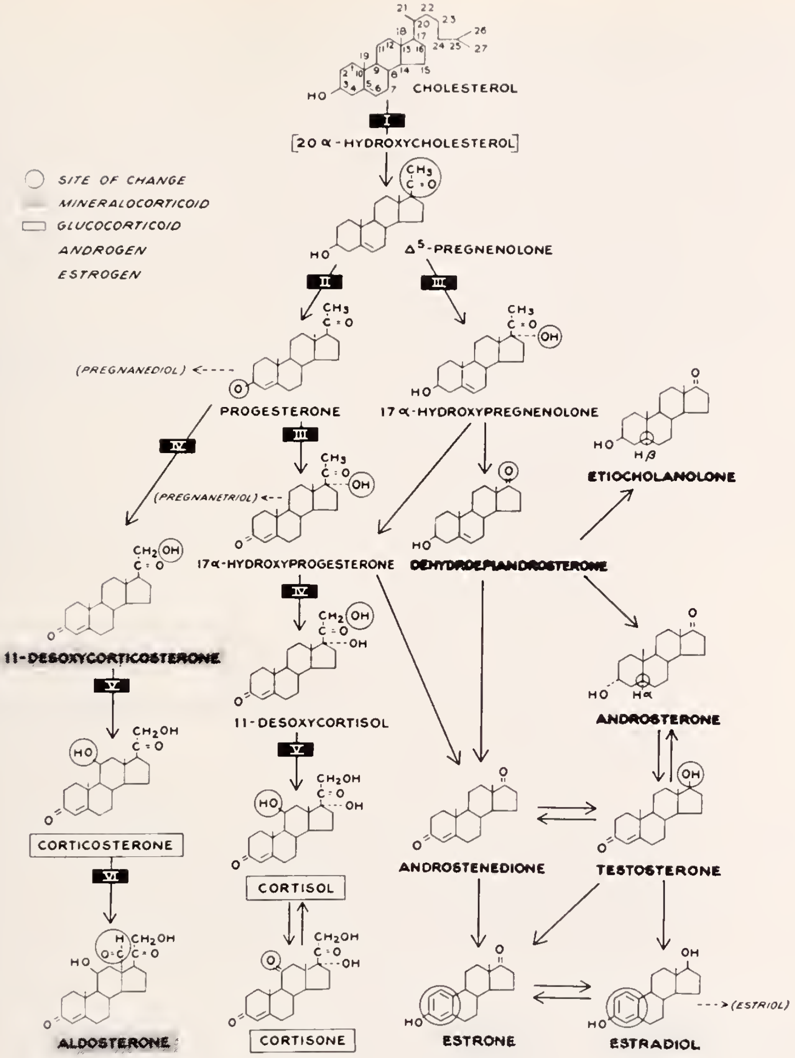
CHOLESTEROL
HO
I
[20α-HYDROXYCHOLESTEROL]
SITE OF CHANGE
MINERALOCORTICOID
GLUCOCORTICOID
ANDROGEN
ESTROGEN
Δ5-PREGNENOLONE
II
III
(PREGNANEDIOL)
PROGESTERONE
17α-HYDROXYPREGNENOLONE
ETIOCHOLANOLONE
IV
(PREGNANETRIOL)
17α-HYDROXYPROGESTERONE
DEHYDROEPIANDROSTERONE
11-DESOXYCORTICOSTERONE
11-DESOXYCORTISOL
ANDROSTERONE
V
CORTICOSTERONE
CORTISOL
ANDROSTENEDIONE
TESTOSTERONE
VI
ALDOSTERONE
CORTISONE
ESTRONE
ESTRADIOL
(ESTRIOL)

FIG. 1. Biosynthetic pathway in the adrenal cortex. (Williams, R. H.: *Textbook of Endocrinology,* 4th Ed. by permission of W. B. Saunders Co.).

into the cortisol pathway since the cells of the zona fasciculata are geared up thereby for the production of cortisol. However, some of the precursor goes into the aldosterone and some into the androgen-estrogen pathway depending on rate-limiting steps which are probably genetically determined. It is likely the ratio of production of aldosterone to cortisol to androgen-estrogen is similar in most healthy subjects and in a "normal" distribution pattern. In congenital adrenocortical hyperplasia, however, where there is a block to the production of cortisol such as occurs with 21 or 11β hydroxylase blocks and cybernetic outpouring of ACTH, there is excessive precursor going into the androgen-estrogen pathway and an adrenogenital syndrome occurs which need have no counterpart in the gonad.

The next question that must be broached is what controls the conversion of androgen into estrogen. It seems likely that the genetic sex, or karyotype controls the differentiation of the undifferentiated gonad in the fetus into either an ovary or testis and also controls the ratio of conversion of androgen (androstenedione and testosterone) into estrogen (estrone and estradiol) (3). It is also possible that gonadotropin or more particularly HCG or LH facilitate this conversion and I shall present the evidence for this later.

We can see that excessive production of androgen by either a tumor or nontumorous hyperfunction of the adrenal cortex or gonad can result in androgenic syndromes (i.e., virilism) in the male or female or similarly excessive production of estrogen by tumor or nontumorous hyperfunction in the gonad or adrenal cortex can result in feminizing syndromes.

Table I illustrates a scheme which I suggested ten years ago (3, 4) based on the concept of the ratio of conversion of androgen to estrogen in order to understand the virilizing and feminizing syndrome. We have been engaged in

TABLE I

*Schema of Virilizing and Feminizing Syndromes**

	Increased androgen (? conversion normal)	Increased conversion androgen → estrogen (with or without increased androgen)	Decreased conversion androgen to estrogen
Male			
Adrenal cortex	Adrenogenital virilism tumor	Prepuberal castration (functional)	
	hyperplasia {congenital acquired	Adrenogenital feminizing syndrome due to tumor or hyperplasia	
Testis	Interstitial cell tumor (child)	Klinefelter's syndrome Interstitial cell tumor in adult (?Sertoli cell tumor)	
Etiology?		?Puberal gynecomastia	
Female			
Adrenal cortex	Adrenogenital virilism tumor	Adrenogenital feminizing syndrome tumor	?Post-menopausal hirsutism
	hyperplasia {congenital acquired	?hyperplasia	
	Stein-Leventhal ovary	?Sexual precocity	
Ovary	Arrhenoblastoma and virilizing ovarian tumors	Tumor granulosa cell	Stein-Leventhal syndrome
	Stein-Leventhal syndrome	theca cell	?Arrhenoblastoma

* Reference 3 in this paper.

TABLE II

Clinical Manifestations of Virilizing and Feminizing Syndromes

Virilizing syndrome	
Male	Female
* Prepuberal	* Virilism
Macrogenitosomia	Hirsutism
Precocious pseudopuberty	Enlargement of the clitoris
	Pseudohermaphroditism
	(Congenital adrenocortical hyperplasia)
Adult	* Virilism
Testicular maturation arrest	Hirsutism
	Enlargement of the clitoris
	Male body build
	Deepening of voice
	Anovulatory menses or
	Amenorrhea
Feminizing syndrome	
Male	Female
* Prepuberal	* Precocious pseudopuberty
Gynecomastia	
* Adult	Adult
Gynecomastia	Postmenopausal bleeding
Loss of libido	
Atrophic testes	

* More overt syndromes.

delineating some of the syndromes and their mechanisms over the course of those years.

Table II illustrates the clinical syndromes that can be seen and some of their manifestations.

It is readily apparent that problems might arise in the characterization or elucidation of hirsutism alone or gynecomastia. It has been our thesis, at least for the past ten years, that the virilizing syndromes reflect an increased ratio of androgen to estrogen and the feminizing syndrome a decreased ratio of androgen to estrogen, i.e., the A/E ratio (or, as we later utilized, the T/E_2 ratio).

I should first like to say something about the virilizing syndrome, confining my remarks chiefly to problems we have investigated and supplementing it by data from other investigations of original or confirmatory nature with emphasis on recent advances.

You are all familiar with the classic virilizing syndrome in the female due to congenital adrenocortical hyperplasia or that due to so-called "acquired" adrenocortical hyperplasia and their differentiation from tumor by the cortisone suppression test. In recent times we have supplemented this by adrenal venography to delineate the presence of adrenal tumor, to localize it and to study its effluent. The next figures (Fig. 2a & b) illustrate two such virilizing benign adrenocortical tumors. Removal of the tumors results in return of the menses and dramatic loss of a good deal of the excessive hair. The latter phenomenon is ordinarily not seen in treated patients with nontumorous virilizing syndromes. Table III shows the content of the adrenal venous effluent in one such tumor compared to the nontumorous side. Preliminary evidence on the study of the adrenal venous effluent would suggest that, unlike the great difference in gradients seen in 1° aldosteronism (5, 6) (Table IV) where the tumor is small, in the large tumor of the adrenogenital syndrome and of Cushing's syndrome the manifestation may be reflected less by the increased concentration of androgen than by the large flow, i.e., volume X concentration on the side of the tumor.

The next problem I should like to touch upon is the problem of hirsutism—Is it a virilizing manifestation and what is its cause?

Until recent years, the biochemical evidence of adrenal or ovarian overproduction of androgen was an increase in the urinary excretion of the neutral 17-ketosteroids. In the absence of this, one could not truly say that isolated hirsutism represented a virilizing syndrome, this in spite of the fact that

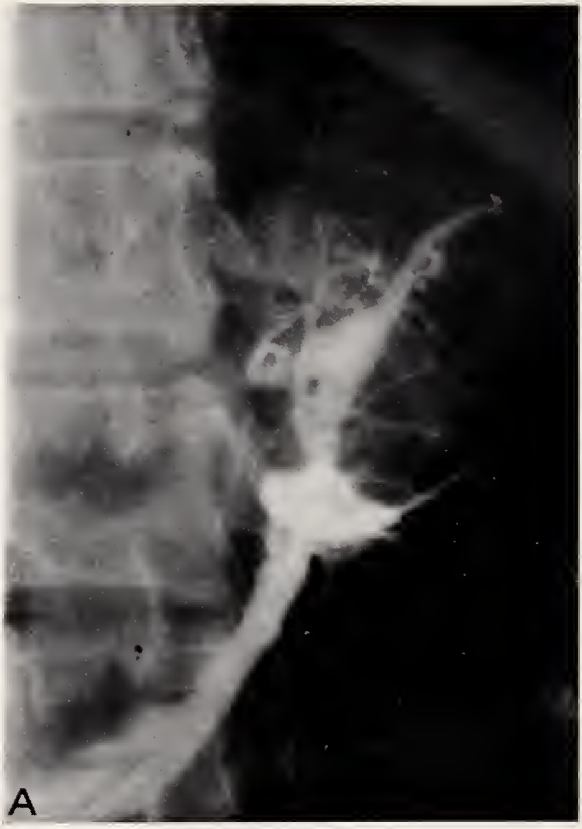

Fig. 2a

Fig. 2 a & b. Virilizing adrenocortical tumors.

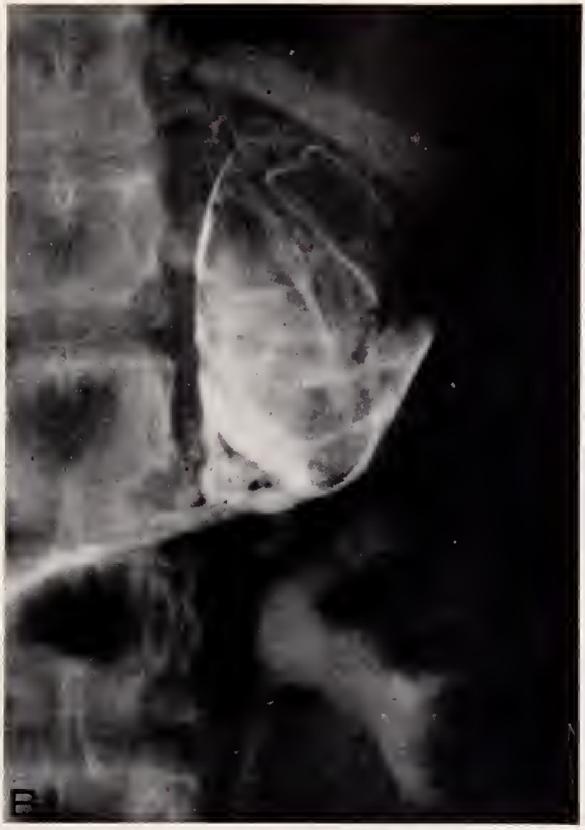

Fig. 2b

TABLE III

Content of Adrenal Venous Effluent from an Adrenocortical Virilizing Tumor

	Testosterone (T) ng/100 ml	Androstenedione ng/100 ml	Estradiol (E_2) ng/100 ml	Androsterone* ng/100 ml	Dehydroepiandrosterone* ng/100 ml
IVC	268.2	860	13.3	39.05	991.5
RAV	299.0	2134.1	5.6	45.1	805.5
LAV (Tumor Site)	438.2	3948	22.9	43.7	2486
Gradient (Side—IVC)	R 22	R 1374	R —	R 6	R —
	L 139	L 3088	L 9.6	L 5	L 1495
L — R	117	1714	17.3	−1	1680

* Sulfate.

adrenal tumors producing only hirsutism as a manifestation had been described and that arrhenoblastomas are usually associated with normal titers of neutral 17-ketosteroids. Indeed, we had shown that the explanation for the latter is the fact that the arrhenoblastomas may produce testosterone in preference to the biologically weak androgens, androstenedione and dehydroepiandrosterone. A

TABLE IV

*Roentgen Findings and Plasma Aldosterone Concentrations ($\mu g\%$)**

Patient	Inferior vena cava	Adrenal vein		T/NT	NT/IVC	Localization by Xrays
		No tumor	Tumor			
F.A.	0.03	0.05	2.99	59.8	1.7	Yes
B.L.	0.18	0.22	6.30	28.6	1.2	Yes
P.M.	0.04	0.07	6.06	86.6	1.7	Yes
R.E.	0.04	0.02	1.10	55.0	0.5	No
S.E.	0.06	0.22	18.86	85.7	3.6	No
C.M.	0.03	0.05	5.37	107.4	1.7	Yes
S.R.	0.02	0.06	1.60	26.7	3.0	No
G.B.	0.06	0.16	3.13	19.6	2.7	Yes
Primary aldosteronism mean	0.06	0.11	5.68	58.67	2.01	
Normal mean (range)	0.015 (0.005–0.02)	0.33 (0.06–1.28)		1.46 (1.03–2.10)	26.0 (8–08)	

* Reference 6 in this paper.

little bit of testosterone goes a long way toward inducing virilism without increasing the urinary excretion of the neutral 17-ketosteroids to which it is converted though it itself is not a 17-ketosteroid.

The introduction of the assay for plasma testosterone introduced a new dimension. Now "idiopathic" hirsutism associated with increased plasma testosterone was no longer "idiopathic" but really a manifestation of a virilizing syndrome. But where did the testosterone come from? The theoretic considerations considered above indicated that it must come from either the ovary or adrenal cortex or both. Two approaches were thus feasible. One, an indirect one, utilizing dexamethasone suppression of the adrenal, followed by ovarian suppression with estrogen or progestational compounds or both or stimulation with HCG, or more directly, measurement of the venous effluent of the ovary and the adrenal cortex. The latter type of study done by Kirschner and Jacobs (7) gave the following findings:

1. The excessive androgens usually came from the ovary, less frequently from the adrenal cortex, and occasionally from both.
2. Surprisingly, dexamethasone at times would suppress the excessive ovarian production of androgen.

This latter finding put a damper on indirect measurements. Nonetheless, in such an indirect study, Rosenfeld and his associates (8) essentially confirmed the findings of Kirschner to the effect that dexamethasone suppressed the elevated free testosterone indices in hirsute amenorrheic women but to values above those in normal women (i.e., there was residual ovarian hypersecretion of androgen). Further in amenorrheic hirsute women suppressed with dexamethasone, HCG induced rises in plasma androgen greater than in normal women or other hirsute women (i.e., indicative of excessive ovarian androgen production).

These findings might suggest that in "idiopathic" hirsutism, if plasma androgen is excessive, it is more likely that amenorrhea will be seen. Eumenorrheic hirsute women had plasma androgen indices above those of normal subjects but they, however, were suppressed by dexamethasone to androgen levels seen in normal women (i.e. presumably the androgen was coming from the adrenal). Ideally, women with hirsutism, if found to have elevated plasma testosterone, should be treated by ovarian suppression by estrogen or progestational compounds or one of the contraceptive pills and by adrenal suppression with a glucocorticoid.

I have touched upon our findings in arrhenoblastomas and I should like to add a word before proceeding to our studies on feminization. In incubation studies we conducted on an arrhenoblastoma we found two important facts (9): 1) the production of testosterone, affording an explanation for the finding of normal urinary neutral 17-ketosteroids in patients with this tumor and 2) the absence of estrogen production by the tumor (Table V), indicating that even in the female, there may be disorders characterized by inability to convert androgen into estrogen. This led our thoughts into disorders that contrariwise might be characterized by excessive conversion of androgen into estrogen.

Chromatin-positive Klinefelter's syndrome is characterized by small testes, gynecomastia, high urinary gonadotropins, a positive Barr body usually due to an XXY karyotype, low plasma testosterone and a typical histologic picture of tubular fibrosis and hyalinization with loss of the spermatogenic elements and increase in the clumps of Leydig cells. We reasoned (3, 4) that, if the ratio of conversion of androgen into estrogen is governed by the karyotype and XY minimizes it and XX facilitates it, what information does XXY convey? (Fig. 3). It would seem likely that the conversion ratio should be between that seen in the male and that seen in the female. On this basis we enunciated in 1963 a

TABLE V

*Incubation Studies on an Arrhenoblastoma (Tissue Slices)**

Compound	Amt. added (mg.)	Radioactivity		Per cent of substrate†
		Spec. activity	Total c.p.m.	
Estrone	10.6	0	nil	0
Estradiol-17β	10.0	0	nil	0
Estriol	12.7	0	nil	0
17-Hydroxyprogesterone	20	12,000	240,000	4.8
Androstenedione	20	2,000	44,000	0.9
Testosterone	20	319	6,380	0.1
20β-Hydroxy-4-pregnen-3-one	10†	271	>2,710	>0.05
20α-Hydroxy-4-pregnen-3-one	10†	2,600	>26,000	>0.5
11β-Hydroxy-4-androstene-3,17-dione	20	0	nil	0

* Savard, K., Gut, M., Dorfman, R. I., Gabrilove, J. L., and Soffer, L. J.: J Clin Endo & Metab 20:165, 1961. By permission of the *Journal of Clinical Endocrinology and Metabolism.*

† Progesterone-4-C^{14}.

Hypothesis as to the biosynthetic abnormalities in Klinefelter's syndrome
Increased conversion of androgen into estrogen

a) Altered genetic sex constitution

XY androgen $\longrightarrow$ estrogen

XXY androgen $\substack{\longrightarrow \\ \longrightarrow}$ estrogen (Klinefelter)

XX androgen $\substack{\longrightarrow \\ \longrightarrow \\ \longrightarrow}$ estrogen

b) Primary increase of gonadotrophins altering conversion ratio
  1) Effect of HCG in normal males in vivo (*Maddock & Nelson* 1952)
  2) Effect in vitro (*Hollander & Hollander* 1958)
  3) Effect of chorionepithelioma (*Martin & Carden* 1963)

FIG. 3. Hypothesis in Klinefelter's syndrome.

hypothesis as to the biochemical defect in Klinefelter's syndrome, to wit, that:

1) there is increased conversion of androgen into estrogen,

2) the increased estrogen and altered estrogen-androgen ratio resulted in the gynecomastia,

3) the tubular changes seen resulted from a decreased local concentration of androgen, and

4) perhaps the increased gonadotropins might represent a primary congenital defect rather than one secondary to testicular changes in structure and function. Incidentally, in November 1973, Capell and his associates (10) published evidence that there is a selective abnormality in the testosterone-LH feedback mechanism in Klinefelter's syndrome.

Some of the supporting evidence was as follows:

1) in Klinefelter's syndrome we found a decreased urinary excretion of neutral 17-ketosteroids and a normal or increased estrogen excretion (4).

2) the administration of chorionic gonadotropin resulted in gynecomastia, and in a modest increase in the urinary excretion of the neutral 17-ketosteroids but a proportionately far greater increase in the urinary estrogen. The histologic picture of the testis to us appeared similar to that seen in Klinefelter's syndrome (11).

3) tubular changes in Klinefelter's syndrome resembled those seen in hypopituitarism (though spermatogenesis may still be seen), those seen following the administration of estrogen, those seen in the testis of patients with feminizing adrenocortical tumor, and those seen following exogenous testosterone administration.

4) the tubular changes in the hypophysectomized monkey could be prevented by local testosterone or large doses of parenteral testosterone and indeed in the human subject, these changes also could be prevented by large doses of testosterone (4).

5) the only proven certain way of producing gynecomastia is by giving estrogen.

These suggestions met with general disbelief. One of the arguments included the statement that it is common knowledge that the breast in Klinefelter's syndrome is fibrotic whereas estrogen causes ductular hyperplasia and a periductular succulent edematous connective tissue.

Meanwhile we were engaged in studies on a man with a feminizing adrenocortical tumor (12) and later a second such subject (13). In brief, we learned the following from these studies:

1) The clinical picture, diagnostic features and clinical course include features seen in Table VI and Figure 4.

2) The plasma testosterone may be normal whereas the plasma and urinary estrogen are markedly increased. And in incubation studies we demonstrated for the first time that the tumor is capable of producing estrogen and that it need not come from peripheral conversion.

3) We were deeply interested in the testicular findings. In general the testis picture in patients with feminizing adrenocortical tumor varies from normal to hypospermatogenesis to hyaline tubular fibrosis and loss of the Leydig cells (Fig. 5). Surprisingly if the tumor is removed, the lesions appear to be reversible and spermatogenesis may return (12–14).

We were particularly intrigued with these findings because of studies we conducted in 1958 (15) in regard to the male adult adrenogenital syndrome.

While reviewing the findings in congenital adrenocortical hyperplasia in the untreated male child we noted that full testicular maturation rarely ensued. At that time we encountered a male with infertility, a decreased sperm count and testicular maturation arrest. The urinary excretion of neutral 17-ketosteroids was 14 mg/24 hours. In view of the fact that the male adult adrenogenital syndrome had never been described and we felt it must exist and in view of the testis findings in congenital adrenocortical hyperplasia, we thought of the possibility that testicular maturation arrest in some instances might represent the

TABLE VI

*Incidence of Clinical Features in Feminizing Adrenocortical Tumors in the Male**

Gynecomastia	98%
Palpable tumor	58%
Atrophy of testis	52%
Diminished libido and/or potency	48%
Pain at site of tumor	44%
Tenderness of breast	42%
Pigmentation of areolae	27%
Obesity	27%
Feminizing hair change	23%
Atrophy of the penis	20%
Elevation of the blood pressure	16%
Increasing skin pigmentation	12%
Varicocele	11%
Acne	8%

* Gabrilove, J. L., Sharma, D. C., Wotiz, H. H., and Dorfman, R. I.: Medicine 44:37, 1965. By permission of *Medicine.*

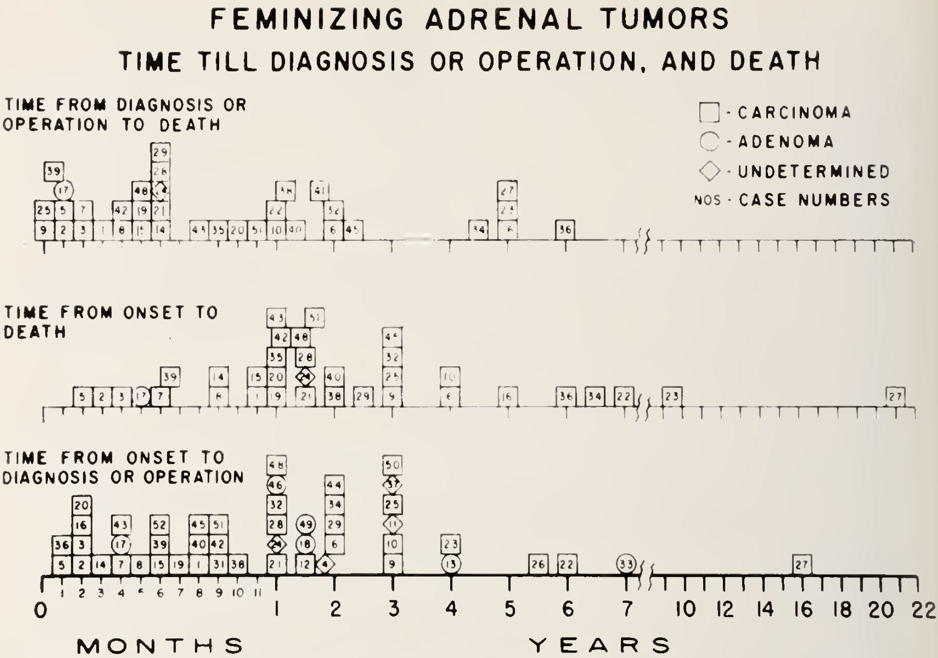

Fig. 4. Prognosis in feminizing adrenocortical tumors. (Gabrilove, J. L., Sharma, D. C., Wotiz, H. H., and Dorfman, R. I.: Medicine 44:37, 1965. By permission of *Medicine*).

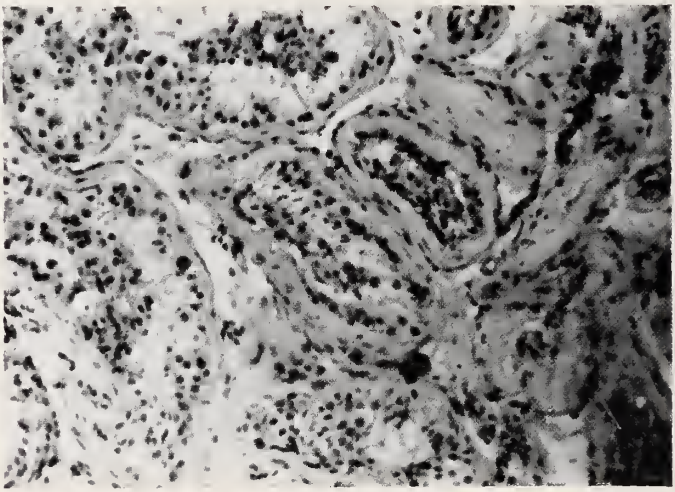

Fig. 5. Testis in patient with feminizing adrenocortical tumor (P.F.).

virilizing male adult adrenogenital syndrome. To test this hypothesis indirectly, we administered cortisone. The sperm count rose to normal. We then stopped cortisone and the sperm count fell. We renewed cortisone and the sperm count again rose to normal and we repeated the testis biopsy. Figure 6 illustrates the

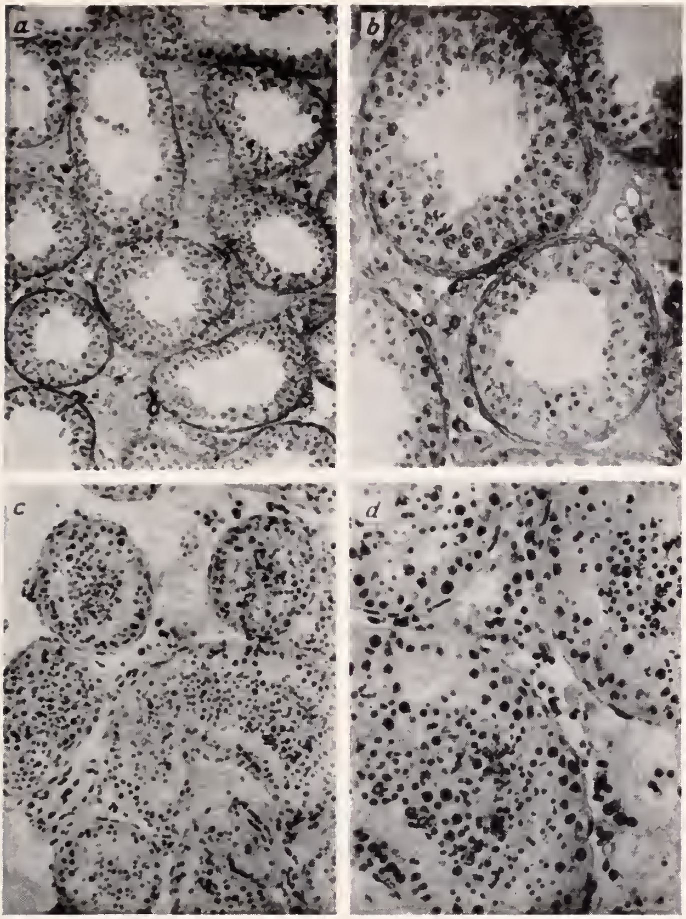

FIG. 6. Testis in patient with male adult adrenogenital syndrome before and after the administration of cortisone (H. H.) (see text), a & b testis prior to and c & d testis after administration of cortisone (Gabrilove, J. L.: Lancet 274:904, 1958. By permission of *Lancet*).

normal histology as compared to the control biopsy. We published this and suggested that testicular maturation arrest might represent the adult male adrenogenital syndrome. Since then several investigations have confirmed these findings.

Now with the observation on feminizing adrenocortical tumors, we recognized that the changes were similar but that probably the testis picture could represent a spectrum rather than only testicular maturation arrest.

Following this we turned out attention to elucidating Klinefelter's syndrome by more refined techniques. In two sets of studies (16, 17) we demonstrated that in chromatin-positive Klinefelter's syndrome there is decreased urinary excretion and presumed decreased production rate of testosterone whereas the urinary excretion and production rate of estrogen is normal or increased indicating an abnormal testosterone-estrogen ratio shifted in favor of estrogen.

Further, in in vitro studies we showed the production of estrogen from testosterone and progesterone is greater in the testis from these patients than in the testes of normal subjects, indicating that the abnormality in conversion occurs in the testis although it is possible, and it remains to be investigated, that it may also occur in the periphery (Tables VII and VIII).

During this period we also investigated our deduction based on embryologic consideration that the adrenal cortex should behave in a fashion similar to the ovary or testis depending on the sex of the individual. We found in incubation studies that the adrenal cortex of the female monkey produces comparatively more estrogen and less androgen than that of the male, analogous to what we find in the gonad (18) (Table IX).

We now turned out attention to gynecomastia (19) with the aid of Dr. Modlinger, an intern taking an elective in my laboratory. We examined the slides of a large group of patients with gynecomastia with the following findings:

The histologic changes in gynecomastia are similar, independent of the associated disease or etiology. Early there is seen ductal hyperplasia and succulent periductal connective tissue ("florid" gynecomastia). After about a year the ducts are squeezed out by fibrosis and the stroma itself becomes fibrotic and hyalinized (Fig. 7). This is particularly well traced in patients with prostatic carcinoma given estrogen.

Incidentally, after we had come to these conclusions, we found some similar observations made by Semb (20) and by Menville (21) many years ago and our studies were therefore confirmatory. In fact, a year or two later, another

TABLE VII

*Urinary Excretion of Testosterone and Estrogen and Estrogen Production Rates in Chromatin Positive Klinefelter's Syndrome**

Patient	Age (a)	Sex chromatin	PR		TG	Urinary excretion		
			E_1	E_2		E_1	E_2	E_3
			(μg/24 h)		(μg/24 h)	(μg/24 h)		
K.T.	17	+	—	12.2	31	10.6	3.6	9.8
G.W.	17	+	22.3	31.2	12	2.5	3.4	2.9
M.S.	17	+	80.2	44.7	20	4.0	1.3	8.8
D.S.	19	+	12.5	29.5	17	6.0	6.5	16.0
C.P.	19	+	39.7	35.8	8	2.5	0.7	5.3
H.K.	29	+	32.9	38.2	24	4.1	2.2	4.0
A.B.	50	+	23.5	13.3	18	9.0	2.0	11.0
Mean			35.2 ± 24	29.3 ± 12.3	19 ± 7.5	5.5 ± 3.1	2.9 ± 1.9	8.3 ± 4.6
Normal subjects (age 23–28 a)			23.0 ± 6.5	31.9 ± 13.2	128 ± 32.5	6.6 ± 3.6	4.3 ± 1.9	15.3 ± 4.2

PR = production rate, E_1 = oestrone, E_2 = oestradiol, E_3 = oestriol, TG = testosterone glucuronide, ± SD.

* Gabrilove, J. L., Nicolis, G. L., and Hausknecht, R. U.: Acta Endocrin 63:499, 1970. By permission of *Acta Endocrinologica*.

TABLE VIII

*Incubation Studies of the Testis from Patients with Chromatin Positive Klinefelter's Syndrome**

Substrates:			*In Vitro* Study			
			[4-^{14}C]T 0.5 μCi 5 μg	[7α-^{3}H] 17α-OH-P 5 μCi; 5 μg		
Subject	Sex Chromatin	Age	Percent Conversion			Ratio × 10
			$OE_1 + OE_2$	$OE_1 + OE_2$	T	
Klinefelter's						
D.S.	+	19	0.71	0.46	0.81	6
M.S.	+	18	0.52	0.19	0.21	9
H.K.	+	29	0.56	0.21	0.23	9
K.T.	+	17	0.32	0.23	0.49	5
R.K.	+	18	0.46	0.25	0.41	6
Controls						
J.E.	–	25	0.41	0.15	3.7	0.4
A.L.	–	59	0.19	0.12	1.1	1
S.L.	–	59	0.15	0.09	1.5	0.6

Incubation studies using [4-^{14}C]testosterone [4-^{14}C]T and [7α-^{3}H]17α-hydroxy-progesterone [7α-^{3}H]17α-OH-P. OE_1 = oestrone; OE_2 = oestradiol; T = testosterone.

* Sharma, D. C. and Gabrilove, J. L.: Acta Endocrin 66:737, 1971. By permission of *Acta Endocrinologica.*

TABLE IX

*Conversion of Steroid Substrates into Estrogens by the Adrenals from Female and Male Rhesus Monkeys**

Substrate		Ratio of products × 10			
		Females		Males	
		No. 5	No. 6	No. 1	No. 2
Progesterone-7α-^{3}H	Estrogens/Androgens	1.5	1.2	0.8	0.8
17α-OH-Progesterone-4-^{14}C	Estrogens/Androgens	1.2	1.0	0.4	0.5
Androst-4-ene-3,17-dione-4-^{14}C	Estrogens/Androst-4-ene-3,17-dione	3.9	4.8	3.1	2.9
Testosterone-4-^{14}C	Estrogens/Testosterone	12.1	11.3	4.5	4.9

* Sharma, D. C. and Gabrilove, J. L.: Comp Biochem & Physiol 31:379, 1969. By permission of *Comparative Biochemistry and Physiology.*

confirmatory study appeared presumably unaware of our findings and those of Semb and of Menville.

The importance to us of these observations were 1) that the arguments based on histologic grounds against estrogen as a causative aspect in the gynecomastia

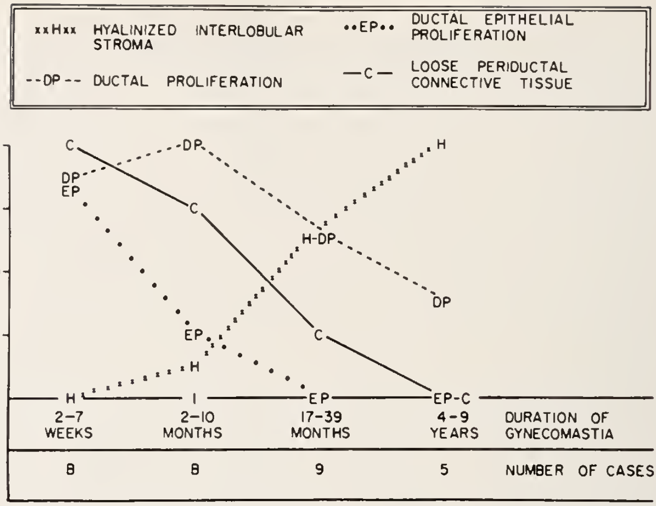

FIG. 7. Evolution of gynecomastia (Nicolis, G. L., Modlinger, R. S., and Gabrilove, J. L.: J Clin Endo & Metab 32:173, 1971. By permission of the *Journal of Clinical Endocrinology and Metabolism*).

of Klinefelter's syndrome were invalid; 2) that possibly all gynecomastia might be explainable on our previous hypothesis of an alteration in the androgen-estrogen ratio or the T/E_2 plasma ratio which we now utilized in our studies. We, therefore, started to investigate the various gynecomastia states from this viewpoint and also attempted to delineate if possible the nontumorous adrenocortical feminizing syndrome in the male and the feminizing tumorous and other nontumorous testicular feminizing syndromes.

We have just reported (22) a young man with gynecomastia, high urinary 17-ketosteroids and high urinary estrogen, a fall in the urinary neutral 17-ketosteroids when he was given cortisone, negative adrenal venography and an abnormal testis on histologic examination. We believe this boy represents a nontumorous feminizing adrenogenital syndrome. Unfortunately our plasma estrogen techniques were not available at the time he was studied. The testis is strikingly reminiscent of that seen in feminizing adrenocortical tumor syndrome.

While searching for the nontumorous feminizing testicular syndrome not due to an overt genetic defect (XXY), we encountered a 28-year-old man with gynecomastia of 6 months' duration. He was the father of two children and had a normal karyotype. No masses were found in the testis. The urinary neutral 17-ketosteroids varied slightly from normal to elevated but the urinary estrogen was increased. The urinary ketosteroids fell when glucocorticoid suppression was carried out as occurs in normal subjects. Adrenal venography was normal. Assay of plasma estradiol revealed it to be elevated, 10 ng/100 ml. It fell on halotestin suppression but not on dexamethasone. The histology of the testis showed the same nonspecific suppression one sees with adrenocortical feminizing tumors. However, repeated biopsies showed the lesions to be progressive and the sperm count fell almost to azoospermia. We were hard pressed for a diagnosis till we succeeded in carrying out selective testicular catheterization. The levels are given in Table X and obviously point to a site in the right testis. Pregnancy tests were normal and plasma gonadotropins were normal. Orchidectomy was carried out with a preoperative diagnosis of a feminizing interstitial cell tumor which it turned out to be. These feminizing interstitial cell tumors are quite interesting. Of approximately 170 interstitial cell tumors, 37 have been feminizing including our own. In the child gynecomastia is associated with macrogenitosomia. The tumors are usually benign. The presenting complaint is gynecomastia. A testis mass may be present but often the patient is unaware of it. Loss of libido and potency occurs but it is a far less important symptom. The hallmark is an increase in plasma or urinary estrogen. The urinary neutral 17-ketosteroid excretion is usually normal in contrast to the increase in feminizing adrenocortical tumors, which are usually malignant. These tumors are of special interest to us for if one examines the histology of the testis, there is seen prepuberally much greater maturation of the testis in areas close to a *virilizing interstitial cell tumor* than one sees at a distance from the tumor and contrariwise in feminizing tumors. In the latter, i.e., feminizing tumor, the most striking degeneration is seen close to the tumor and less at a distance. This evidence plus the recent publication of Heller et al (23) as the effects of testosterone on the testis afford support for our ten-year old contention that the local concentration of testosterone is a vital factor in tubular maintenance (3, 4, 24, 25).

Interstitial cell tumors can be malignant but interestingly those with gynecomastia at the time of diagnosis are benign even though patients with nonfeminizing interstitial cell tumors at the time of diagnosis may later develop gynecomastia when metastases are present.

The production of estrogen by feminizing interstitial cell tumors has been demonstrated in vitro by Kliman et al (26) but more importantly the secretion of estrogen by the tumor is demonstrable in vivo by selective testicular catheterization (26, 27, personal observation). There one sees the abnormality in T/E_2 ratio in the periphery and in the testicular secretion in association with these tumors. Removal of the tumor results in a return to normal of the plasma estradiol and testosterone titers, regression of the gynecomastia and a return to normal of the sperm count. I indicated before that HCG may be acting to increase the testicular conversion of androgen to estrogen. I quoted the earlier work of Maddock and Nelson (11) and further support is afforded by the data of Weinstein et al (28) on the testicular effluent and peripheral plasma after the administration of HCG to normal subjects. In the spermatic vein effluent of these patients, androstenedione, dehydroepiandrosterone and testosterone rose 3 to 5 times whereas estradiol increased eight- to ninefold suggesting a distal effect on the biosynthetic pathway. There was also a marked increase in peripheral plasma estradiol in our patient with the interstital cell tumor (×5) as contrasted to the modest increase in plasma testosterone (11).

In our previous publications we indicated our belief that gynecomastia may

TABLE X

Plasma Testosterone/Estradiol Ratio in some Disorders Associated with Gynecomastia

Patient	Diagnosis	ng/100 ml E_2	ng/100 ml Testosterone	T/E_2
K.B.	Feminizing Interstitial Cell Tumor	9.8	392	40
		9.8		After dexamethasone 2 mg
		10.2		After dexamethasone 8 mg
A.G.	Drug gynecomastia	4.7	486	103
J.H.	Gynecomastia	3.7	517	140
N.H.	Chromatin neg. Klinefelter's syndrome with gynecomastia	2.1	176	84
R.K.	Puberal Gynecomastia	1.7	219	129
W.P.	Cirrhosis, gynecomastia	3.9	305	78
R.W.	Alcoholism, gynecomastia	3.4	233	69
J.M.	Alcoholism, infertility	2.5	147	59
Mean Normal				
Literature		2.3	709	308
Personal Series		2.9 (n = 32)	480 (n = 22)	163
K.B.	Feminizing Interstitial Cell Tumor			
	Right Spermatic Vein (Tumor site)	551	13600	
	Left Spermatic Vein	16.4	1220	
	Inferior Vena Cava	6.8	143	

result from alteration in the androgen-estrogen ratio (3, 4, 12, 16, 17, 19). Table X illustrates some preliminary supporting data that we submitted in one of our NIH applications. Since that time a group of confirmatory papers, if not a flood, have appeared. These have embodied data on the gynecomastia seen in thyrotoxicosis (29), in cirrhosis of the liver (30), in puberal gynecomastia (31), and in that seen in association with digoxin therapy (32). It has been known that marked feminization occurs in the feminizing testis syndrome where, in genetic males, with normal male amounts of testosterone, which is apparently not biologically effective, normal male amounts of estrogen produce perfectly feminized "men."

Thus, in summary, evidence is accruing that alteration in the normal androgen-estrogen ratio is important in our understanding of virilizing and feminizing syndromes, that abnormalities in these ratios can adversely affect the testis and the ovary and can induce gynecomastia. An overall view from this perspective permits one to view the sexual disorders of the gonads and the testis in a more rational and understandable light.

Although abnormalities have been demonstrable in the adrenal and gonadal secretion of androgen and estrogen, work still remains to be done on how significantly alterations in these ratios may result from the conversions known to occur in the peripheral blood (33) or secondary to abnormalities in the metabolic clearances (33) or in the sex hormone-binding globulin.

Acknowledgment

These studies were carried out in large measure with the collaboration of Drs. Dinesh C. Sharma, Herbert H. Wotiz, Giorgio L. Nicolis, Arthur R. Sohval, Robert S. Modlinger, and Harold A. Mitty.

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Neural Origin of Granular Cell Tumor

CYRIL TOKER, M.D.

Abstract

Origin of a recurring, invasive, granular cell tumor from the ulnar nerve is described. The lesion was seen to develop within the substance of the nerve trunk, and granular cells surrounded the axonal fibers. The granular cells were, presumably, of Schwannian derivation. The tumor has exhibited both histologic and clinical stigmata of malignancy. The neural origin of some granular cell tumors can not be denied.

Evidence in support of a neural origin for granular cell tumors has accumulated. The issue is, however, still debated (1). The ensuing brief report is presented in order to substantiate the view that at least some granular cell tumors are, undoubtedly, of neural derivation.

Clinical Data

At the age of 21 this white female noted decreased muscular strength and atrophy of the left hand. An anterior transposition of the ulnar nerve at the elbow, was performed. She apparently responded to this procedure. Eleven years later, at the age of 32, she presented with pain in the hand, of ulnar distribution. At operation the deep branch of the ulnar nerve was found to be thickened. A firm, cylindrical, segment of the nerve, measuring 6.0 by 0.6 cm, was resected.

Microscopic Pathology

Section of the affected segment revealed granular cell transformation within the substance of the nerve fibers (Figs. 1, 2). The process could be traced from localized areas of involvement (Fig. 1) to a more complete and confluent replacement of the entire circumference. At higher magnification granular cells could be seen to envelop the axonal fibers (Fig. 2).

Four years subsequent to the resection, at the age of 36, the patient again manifested pain at the operative site. A recurrent mass measuring 4.0 × 3.0 × 2.5 cm, was removed. The tumor showed invasion of adjacent structures and displayed, histologically, the features of a malignant granular cell tumor (Fig. 3). Nuclear hyperchromasia, pleomorphism, and mitotic activity were evident. Local resection was performed. Seven months later massive recurrence with bony involvement necessitated amputation through the mid-forearm.

Comment

A neural, presumably Schwannian, origin of the lesion under consideration, cannot be disputed. Location within the nerve sheath was manifest, and has been illustrated above.

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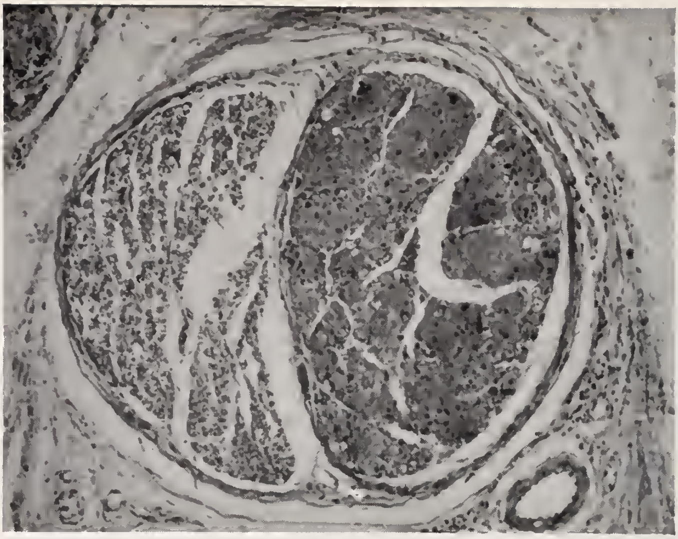

Fig. 1. Low power micrograph of nerve fiber showing granular cell transformation of the right half (H&E × 80).

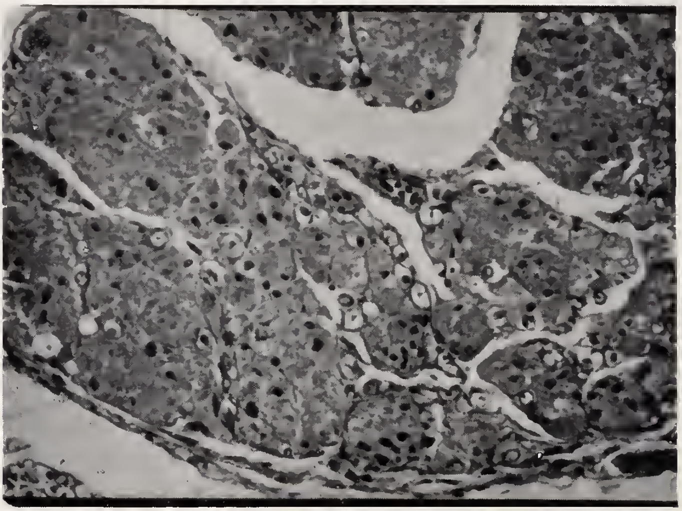

Fig. 2. High power micrograph showing granular cells within the substance of the nerve fiber. Axons are visible as lightly staining round structures within their Schwannian sheaths. The granular cell nuclei show intense hyperchromasia and they vary in configuration (H&E × 200).

It is not our purpose to review the literature on this subject, which has been adequately summarized by Sobel et al (1). We would, however, hesitate to ascribe a neural origin to all granular cell tumors. Thus the development of granular cell tumors in the cervices of new born mice after estrogenic stimulation (2) would suggest a myogenic origin in some instances. The growth of a

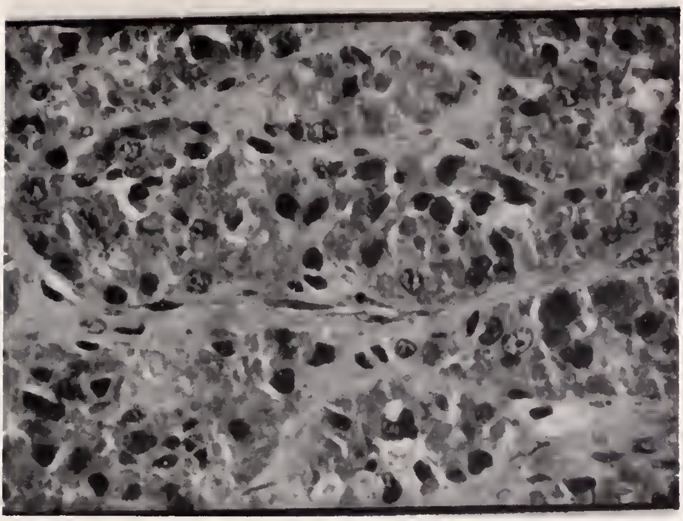

Fig. 3. High power micrograph of recurrent tumor. The granular cells exhibit hyperchromasia, pleomorphism, and mitotic activity (H&E × 320).

tracheal granular cell tumor during pregnancy (3) may have similar implications. It may, therefore, reasonably be suggested that granular cell tumors may derive either from muscular or neural elements.

The lesion described herein exhibited cytologic features of malignancy, both within the original and recurrent growths. Extensive invasion of palmar structures occurred. Thus far no evidence of remote involvement has been detected, but a period of only a few weeks has elapsed subsequent to the most recent surgery.*

Acknowledgment

The author is indebted to Dr. Bernard E. Simon for his permission to publish this report.

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* Six months subsequent to amputation, multiple axillary node metastases developed and were treated by block dissection.

Cryohemorrhoidectomy

CHANGYUL OH, M.D. AND DAVID A. DREILING, M.D.

Unbearable post-hemorrhoidectomy pain has deterred many patients from seeking qualified medical aid and driven them to nostrums advertised in popular magazines, radio and television. Recognition of this fact prompted us to evaluate a new painless technique, cryogenic hemorrhoidectomy (1), which can be performed on an ambulatory basis without anesthesia or hospitalization. This report is based on the early results of 50 cases subjected to cryogenic hemorrhoidectomy. (Since the time of this report an additional 100 cases were operated on by this method.)

Clinical Materials and Methods

Fifty patients were subjected to cryohemorrhoidectomy, 26 men and 24 women, ranging in age from 29 to 82, an average of 51. Eight patients had first-degree hemorrhoids, 27 had second-degree and eight third-degree; the remaining seven had external hemorrhoids and did not experience preoperative bleeding. All patients had a preliminary proctoscopy and sigmoidoscopy and, where indicated, a barium enema examination.

The patients were brought to the ambulatory unit and those who were anxious were given 10 mg of Valium® and 50 mg of Demerol I.M.® approximately 20 minutes before the operation. The patient was then placed on the operating table in the jack-knife position and his buttocks spread with adhesive tape. Five percent Xylocaine® ointment was rubbed into the anal canal. Five minutes later the patient was asked to bear down and the hemorrhoids were identified.

The cryosurgical system was set up by simply plugging the cryoprobe to the console.* This system, nonelectric and operating on nitrous oxide, produces controlled temperatures down to −89°C. The hemorrhoids were frozen by a technique varying with the type or degree of hemorrhoids. External hemorrhoids and first-degree or early stages of second-degree hemorrhoids were frozen directly for three to five minutes and occasionally repeated a second time for the larger hemorrhoids. Advanced second-degree and third-degree hemorrhoids were ligated with rubber bands, after which the tufts were frozen. In cases of excessive anal tags or anal papillomas, a third method was used. The tissue was frozen, following which the pedicle was ligated where the lesion was located at the sensitive anodermal region.

Patients were returned to the ambulatory unit, where they remained from a few minutes to a few hours, following which they were discharged with a prescription of 5 mg Valium or Tylenol®. All patients were seen postoperatively at one week, three weeks, six weeks, and less frequently thereafter. The patient

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* Frigitronic of Connecticut, 770 River Road, Shelton, Connecticut 16484.

was warned that he might have a moderate anal discharge for about two weeks and would feel urgency of bowel movements for a few days. No specific diet was necessary, but laxatives were often required immediately postoperatively. Daily Sitz baths were required for a few weeks, particularly for those who had external hemorrhoidectomy. The patient was told he could return to his regular duties as soon as he felt comfortable enough to do so.

Clinical Features of Cyrosurgery

Characteristically, a cryolesion is a relatively painless, bloodless, slowly progressing, sharply demarcated ulcer that heals rapidly with little scar formation. Soon after freezing the external hemorrhoids, they become swollen and appear hemorrhagic. The degree of edema depends on the condition of the anal skin: i.e., loose skin displays more pronounced edema (Fig. 1), while thick skin is less edematous (Fig. 2). The edema gradually increases until the third day, accompanied by a considerable discharge of exudate, which continues until the necrotic tissue separates from the base. The color of the skin becomes mottled with pale- and dark-bluish spots, with a sharp demarcation on the border zone, and undergoes gangrene between the first and fourth days. The necrotic hemorrhoid tissue separates from the underlying base in one to two weeks. The ulcer thus formed is gradually filled with clean granulating tissue which rapidly progresses to healthy scar formation by the third to sixth week (Fig. 1 & 2). A parallel observation was made in experimental studies in the dog (Fig. 3).

The postoperative course of cryosurgery for internal hemorrhoids is even

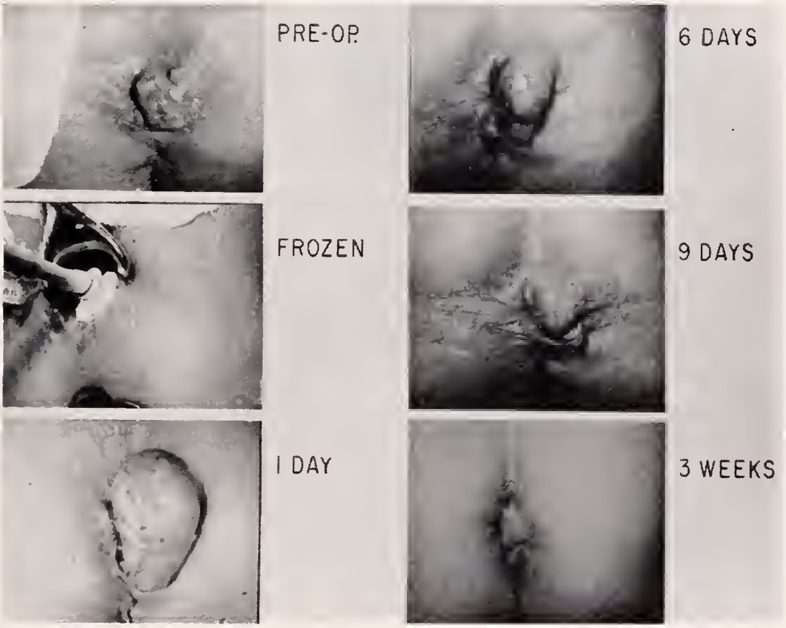

FIG. 1. Clinical course for the prolapsed internal hemorrhoid. Note the marked edema on the first postoperative day.

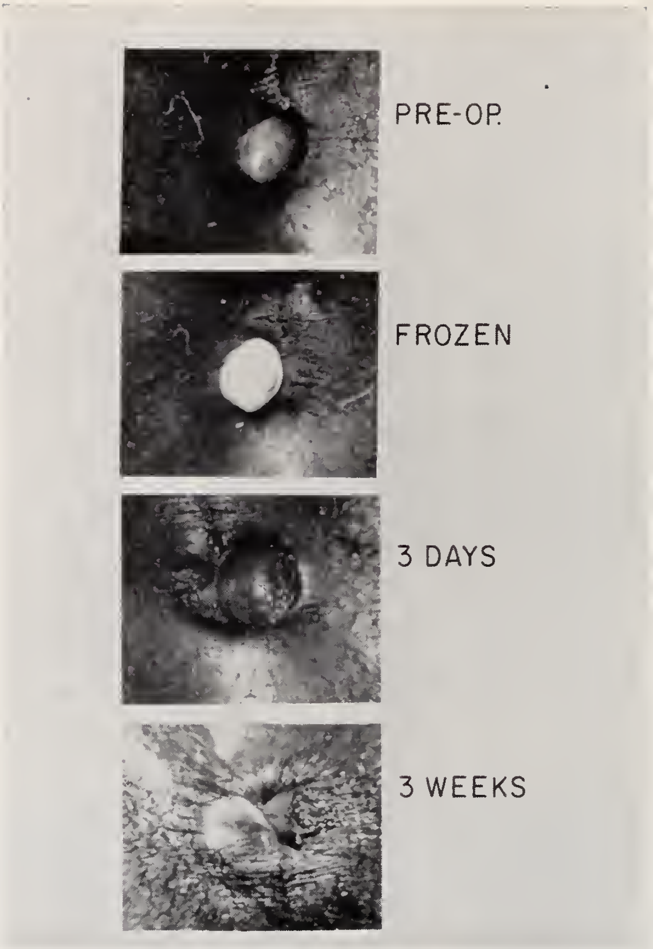

Fig. 2. Clinical course for the external hemorrhoid. Note slight edema on the third postoperative day.

smoother than that for external hemorrhoids. This observation was made by digital examination, since inspection by instrumentation is apt to produce trauma to the friable anal mucosa. All patients were examined with a proctoscope by the sixth week and healing with minimal scarring was found, except for a few patients in whom a granulating base persisted for several additional days. Thus the process of healing following internal hemorrhoidectomy is essentially the same as that for external hemorrhoids, except for occasional prolongation. Clinically less discharge and less discomfort was encountered in the postoperative course following treatment of internal hemorrhoids than that of external hemorrhoidectomy. The prolapsed hemorrhoids were converted to nonprolapsed hemorrhoids by rubber band ligation in order to simplify treatment and make the postoperative course smoother. The resulting tuft of hemorrhoids, then

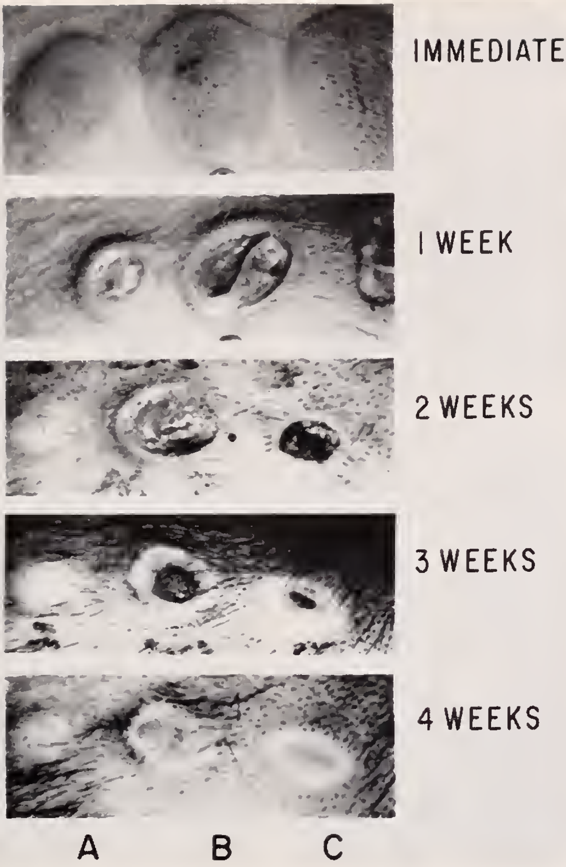

Fig. 3. Local effect of freezing on dog skin: a) Frozen for three minutes. b) Repeat freezing for three minutes. c) Frozen for five minutes.

frozen, will shrink completely within 48 hours and slough with varying degrees of hemorrhage by the third to seventh day.

Results

All patients were followed for a nine- to eighteen-month period. No anal incontinence, stricture or ulcer was found during this time. Wounds were well healed within three to six weeks with little scar formation. Pain was minimal, and most patients needed no more than a mild analgesic such as Tylenol. Four patients required a few tablets of Demerol for 24 to 36 hours following surgery. However, approximately half of the patients were symptom-free while the other half complained of mild to moderate discomfort for a few days. Four patients hemorrhaged, three to a minor degree, the other severely. The latter, an 82-year-old man bled on the eighth postoperative day and was admitted to a local hospital. Examination, except for mild oozing from the wound, failed to locate

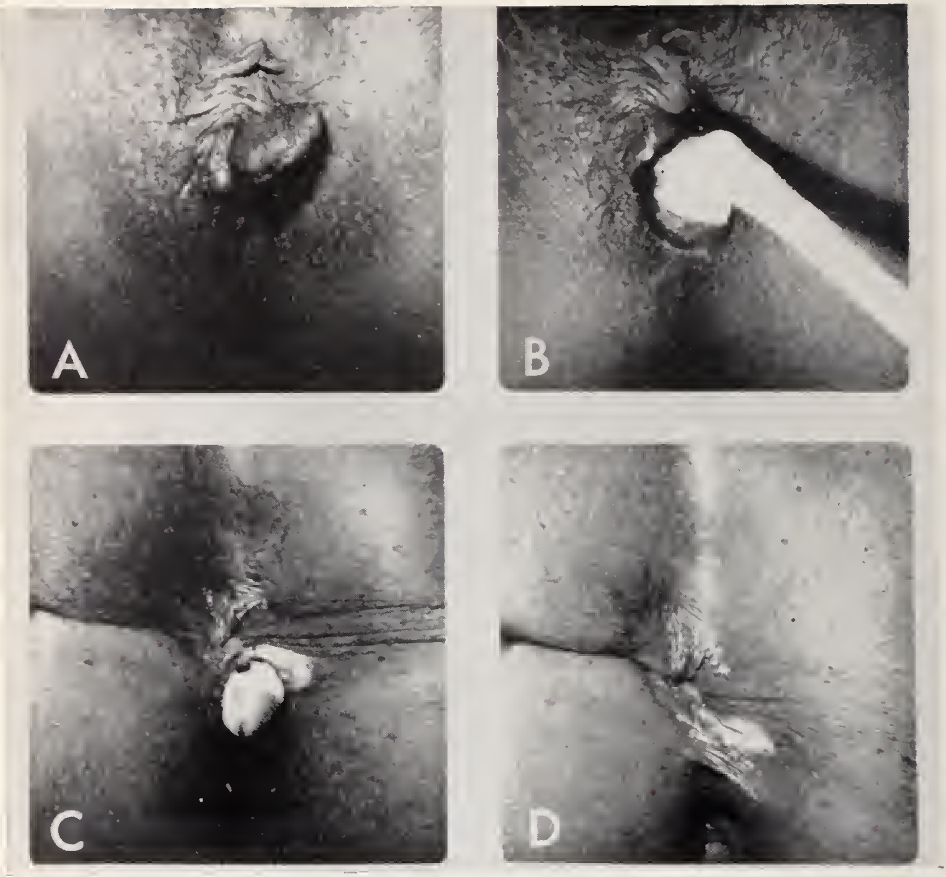

FIG. 4. Clinical course of cryosurgery for anal papilloma: a) Prolapsed papilloma. b) Freezing the papilloma. c) Pedicle of papilloma is ligated at three weeks post-freezing because of intact pedicle. d) Two weeks following ligation.

the source of the bleeding. Multiple diverticula, however, were found in the descending colon on subsequent barium enema. The bleeding required two units of blood and ceased spontaneously 24 hours after hospitalization.

Three patients presented for incomplete hemorrhoidectomy but one patient required repeated procedures for the persisting symptoms. The hemorrhoids of two patients, who had large anal papillomas, failed to be eradicated by simple freezing and required banding three weeks after the initial procedures (Fig. 4). Fourteen patients healed with asymptomatic anal tags.

All patients returned to full activity within 15 days following surgery. The average return-to-duty time was five days: 28 within three days, 15 within seven days and seven by the fifteenth day (Table I).

Discussion

Numerous methods for the treatment of hemorrhoids have been described in the literature, including injection, ligation, dilation and surgery. The injection

TABLE I

Comparison of the Results of Hemorrhoidectomy

Method:	Surgical Excision	Cyrosurgery (50 Cases)	
Author:		Lewis (1972)	Oh (1973)
Pain (%)	33–90	4	8
Hospital Stay (days)	3–8	0	0
Disability (days)	14–30	1	5
Urinary Retention (%)	0.5–32	0	0
Skin Tags (%)	30–34	4	28
Fissure (%)	0.5	0	0
Recurrence (%)	1–3	2	6
Minor Incontinence (%)	3	0	0
Bleeding (%)	3–4	8	8*

* One patient: hospitalized.

and rubber band ligation techniques are limited to selected internal hemorrhoids (2, 3). Forceful dilation of the anus may relieve the symptoms but hardly eradicate pathology (3, 4). The surgical treatment remains the most reliable and effective therapy for hemorrhoidal disease, despite the drawback of unbearable postoperative pain, a fact that occasionally stimulates various modifications of operative technique with indifferent success. Cryogenic hemorrhoidectomy was proposed by Lewis and associates in 1969 (1) to remedy the problem of pain. They reported gratifying results with their initial 50 patients and were convinced by their experience that cryosurgery is equal or superior to those of surgical excision. Subsequent follow-up studies were most favorable (5, 6) and their results have been confirmed by others (7–9).

Arteries and veins are resistant to cryonecrosis, accounting for the ineffectiveness of cryosurgery for the highly vascular lesions such as the tonsils. Large prolapsed third-degree hemorrhoids are also less effectively destroyed by freezing. Obviously, the greater the vascularity of the hemorrhoid, the greater the cooling power required to freeze it. However, when circulation is blocked by ligation, as suggested by Barron (8), the large prolapsing hemorrhoids can be effectively frozen and successfully treated. With the combination of ligation and cryosurgery, we have managed all types of hemorrhoids without difficulty, even those large prolapsed hemorrhoids that were excluded by Lewis (5).

Another distinctive fact is that little pain is felt during the application of the cryoprobe to the sensitive anal skin. It is interesting that after the first cycle of freezing and thawing, the anal skin is so insensitive to pain that ligation of the anal papillomas or tags is possible. Such a sequence, as demonstrated in two cases of this series, is a useful method for the treatment of large broad-base papillomas, which often resist cryodestruction. The disadvantage of ligation and cryosurgery, however, is the frequent occurrence of bleeding when the tuft is sloughed off from the base.

The cryolesion is unique and characteristic in its clinical course: the formed iceball becomes a punched-out ulcer and heals rather rapidly with little scar formation between three and four weeks. During this process, pain is minimal, inflammatory reaction is negligible, and bleeding is almost absent from the wound.

Our results confirm those of Lewis and others, and our comparison of standard surgical excision with cryohemorrhoidectomy indicates that the cryosurgical procedure has a definite advantage over the surgical method (Table I) (5, 10–12). There are, however, minor details of the technique that need to be improved in order to avoid the untoward complications.

Summary

Cryogenic hemorrhoidectomy has been evaluated in 50 patients. These early results indicate that this technique is superior to standard surgical procedures in terms of postoperative pain and equally effective in eradication of hemorrhoids. Its advantages are: 1) it is an ambulatory procedure; 2) no anesthesia is required, except for local application of Xylocaine ointment; 3) the postoperative course is smooth and relatively painless and the wound heals rapidly.

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Postoperative Combined Undermining Infection of Abdominal Wound due to Yersinia Enterocolitica

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Yersinia Enterocolitica, also known as Pasteurella Pseudotuberculosis X has been recognized as the cause of infection in animals since the last century. During the first half of this century, a number of infections have been reported in man. As noted by Sonnenworth and Weaver (1), there has been a steady increase in the incidence of infection due to this small organism, particularly over the last few years. The literature records an extremely wide range of clinical syndromes in which this rather unusual organism has been isolated, including splenic abscess, gastroenteritis, mesenteric lymphadenitis, cholecystitis, catheter infection, and occasionally even appendicitis. This report describes an unusually virulent wound infection, a "Meleney ulcer," resistant to antibiotics.

Case History

A 39-year-old woman patient was admitted to The Mount Sinai Hospital with right lower quadrant pain of twenty-four hours' duration. On examination, she was found to have right lower quadrant tenderness with some guarding, but little spasm. The symptoms increased in severity, and a positive Rovsings' sign became apparent. Her temperature was 99.8°F, her pulse rate 96 per minute, and the white blood cell count 9,500.

The local findings persisted and indicated an intraperitoneal inflammatory process. Laparotomy was performed through a transverse right lower quadrant muscle splitting incision, which was extended medially towards the midline after opening the peritoneum. The operative findings were a right large, tense, twisted, blue pregangrenous hydrosalpinx with serosal inflammation of the adjacent appendix. The right tube and appendix were removed without difficulty. The postoperative course was uncomplicated for the first week. On the tenth postoperative day, however, a wound infection developed; the wound was opened to allow drainage of 40 cc of yellowish-white pus. Despite the administration of tetracycline, and staphcillin, the wound infection persisted. Cultures obtained revealed Yersinia Entercolitica plus staphylococcus aureus. For four weeks following the first procedure, the wound, which was now obviously undermined continued to discharge considerable amounts of sloughing, necrotic, fatty tissue and pus. Since there was no resolution of infection on antibiotics, a wide incision and drainage was necessary and was performed. At the second procedure, the wound was laid open, and numerous deep undermining sinus tracts radiating from the wound in all directions and containing a somewhat mucoid material were found. The wound, having been laid open together with all undermining sinus tracts, was packed with iodoform gauze. Following this, the wound healed slowly, taking a total of another six weeks before complete closure. The patient has been discharged from the hospital in satisfactory condition, with only a narrow scar remaining.

Discussion

Although Yersinia Enterocolitica has been described as a cause of numerous infections, there is no report of this organism being found in association with

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staphylococcus aureus in a human wound infection. The source of the organism was most probably the appendix in which this organism may be found. There was, however, no obvious inflammation of this organ other than subserosal inflammation related to the adjacent hydrosalpinx. The tube was distended to a diameter of approximately five centimeters, and completely twisted one and one-half rotations at its points of attachment proximally and distally. It is possible that the tube was the source of this unusual organism. However, in a study of Nilehn and Sjostrom (2) in which 92 of 93 patients were cultured, Yersinia was found to be present in eight of 10 cases with acute regional terminal ileitis, in nine of 60 with mesenteric lymphadenitis, and only two of 335 with acute appendicitis.

The interesting feature about the 21 patients reported with Yersinia Enterocolitica, 18 with positive cultures and three with positive serology, is that none of these patients went on to develop true Crohn's Disease. Thus, Yersinia-positive regional enteritis would seem to be a different disease from the Yersinia-negative acute episode which goes on to true Crohn's Disease in a significant proportion of cases. Five of the patients in whom Crohn's disease was microscopically verified went on to further complications and required resectional surgery within the next few years. None of these patients had positive cultures or serology.

Thus, it would seem that this strange organism is associated with a benign form of acute terminal ileitis, and also with mesenteric lymphadenitis in a fair number of patients. The diseases associated with this organism enumerated above do not include synchronous-combined infection with staphylococcus aureus causing undermining ulceration of an abdominal wound.

Summary

A patient with a combined undermining wound ulceration similar to the synchronous-combined Meleney ulcer has been described. A positive culture of staphylococcus aureus and Yersinia Enterocolitica was obtained. This infection was resistant to antibiotics and ultimately required a complete secondary incision and drainage of the entire wound area. It is important that surgeons and bacteriologists be aware of this organism which is being recognized more frequently today, and to which an ever wider spectrum of disease in a man is attributed.

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Parathyroidectomy for Secondary Hyperparathyroidism

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Hyperplasia of the parathyroid glands is recognized as a constant physiologic response to chronic renal failure. This is evident in the greatly increased size and weight of parathyroid glands in patients dying of uremia (1), as well as in the significant increase in circulating parathormone in the serum of those known to have chronic renal disease (2).

In earlier years, patients afflicted with renal failure rarely survived long enough for hyperparathyroidism to become a problem. Since the advent of chronic dialysis and renal transplantation, useful life has been prolonged for many of these people. As a result, symptoms attributable to secondary hyperparathyroidism are now encountered with increasing frequency.

One of the most common problems seen in patients maintained on chronic dialysis is renal osteodystrophy. When this is progressive and incapacitating, Stanbury et al (3) and others have clearly demonstrated the efficacy of parathyroidectomy. In other reports, parathyroidectomy has been advocated in those with hypercalcemia, soft tissue calcification, or pruritis. This review of our experience attempts to clarify the indications and expectations of surgery for secondary hyperparathyroidism.

Material

Charts of all patients treated for parathyroid disease from 1938 through 1971 were reviewed. These included those seen on the surgical service of the City Hospital Center at Elmhurst as well as at The Mount Sinai Hospital. The diagnosis of secondary hyperparathyroidism was verified in 9 of 10 patients in whom it was recorded.

Case Reports

Case 1: Our first patient, a 45-year-old woman, was explored in 1942. In addition to a long history of chronic glomerulonephritis and hypertension, she had diffuse bone demineralization and cystic changes. Biopsy of a clavicular cyst had revealed a "brown tumor of hyperparathyroidism." Four enlarged parathyroids were identified and chief cell hyperplasia was confirmed in the 2½ glands which were excised. Postoperative hypocalcemia and progressive uremia led to the patient's death within a month.

Case 2: A 26-year-old woman was admitted with a BUN of 80 mg %, bone pain, known hypertension for three years, but no history of urinary calculi. Renal biopsy showed evidence of chronic glomerulonephritis. Radiographs revealed diffuse bone demineralization. At surgery, in May 1958, two enlarged parathyroids showing chief cell hyperplasia were removed. Postoperative calcium levels declined, ranging between 7 and 8.8 mg %. Hyper-

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tension persisted and bone pain showed some improvement, but she was lost to follow-up after one year.

Case 3: This 40-year-old woman was explored in October 1962 because of symptoms related to multiple cystic bone lesions. She had been hypertensive for eight years and was known to have chronic pyelonephritis for at least six years. All four parathyroids were enlarged and 3½ glands showing "hyperplasia" were removed. Serum calcium, which had been 8 to 9 mg % preoperatively, dropped as low as 4 mg % and the patient died on the nineteenth postoperative day as a consequence of tetany and progressive renal failure.

Case 4: A 57-year-old woman had neck exploration in May 1966, seven months after excision of a "giant cell tumor" of the mandible. Hypertension and albuminuria, ascribed to chronic pyelonephritis, had been present for 14 years. Four enlarged parathyroids were identified. Both lower glands and one-half of each upper gland were excised and showed chief cell hyperplasia. Chronic dialysis was started four years later because of continued deterioration in renal function. Within 18 months, pruritis and symptomatic osteodystrophy culminating in a pathologic fracture of the femur led to reexploration and removal of all remaining parathyroid tissue. Bone pain was relieved and the fracture healed, but the pruritis persisted.

Case 5: A 38-year-old woman with known polycystic kidney disease presented with a BUN of 142 mg %, calculi in both renal collecting systems and ectopic soft tissue calcification. Serum calcium ranged between 9.6 and 10.6 mg %, despite a serum phosphorus from 10.2 to 13.5 mg %. At surgery, in November 1967, 3½ glands showing clear cell hyperplasia were excised. The patient died four months later as a result of progressive renal failure.

Case 6: A 54-year-old man was placed on chronic dialysis in 1966 following a one-year history of azotemia attributed to chronic glomerulonephritis. Progressive bone demineralization ensued and neck exploration was performed in January 1969 because of a pathologic metatarsal fracture and diffuse bone pain. A mass of parathyroid tissue thought to represent a fusion of the upper and lower glands was removed from each side. The pathology report was clear cell hyperplasia and the patient remains on dialysis with no osseous symptoms.

Case 7: A 41-year-old woman was explored in June 1969 because of symptomatic osteodystrophy, including a pathologic fracture of the tibia six months previously. She had been azotemic for nine years and gave a long history of hypertension, albuminuria, and anemia. Resection of 3½ glands was performed and hyperplasia was evident histologically, with all three cell types represented. She was started on chronic dialysis three months later and has since had no radiographic progression or symptoms of osteodystrophy.

Case 8: This 59-year-old woman with chronic pyelonephritis had been on chronic dialysis for eight months because of chronic renal failure associated with hypertensive cardiovascular disease, when she developed increasing discomfort related to osteodystrophy, as well as pruritis. At surgery, in June 1971, three glands showing chief cell hyperplasia were excised. Bone pain disappeared, but the pruritis improved only slightly.

Case 9: A 61-year-old man was explored in July 1971 because of severe pruritis. He had been on chronic dialysis one year for chronic renal failure associated with arteriosclerotic cardiovascular disease. Osteodystrophy, although present, was not symptomatic. Removal of 3½ glands showing clear cell and oxyphilic cell hyperplasia did not significantly improve the pruritis.

Comments

Indications: Renal osteodystrophy was present in eight patients and was symptomatic in all except one. Pruritis was the reason for parathyroidectomy in the latter patient and was a significant problem in two others. One patient had surgery because of progressive soft tissue calcification associated with serum phosphorus levels as high as 13.5 mg %.

Rapidly rising serum calcium levels were not observed in any of our patients. Rather than invoke the concept of "tertiary hyperparathyroidism," it seems more likely that such hypercalcemia is directly related to the duration and severity of the renal disease. According to this view, so-called "autonomous" function merely indicates that hyperfunctioning parathyroid tissue exceeds a certain critical mass. This is consistent with the observation that serum calcium levels will usually return to normal in patients who have undergone renal transplantation after subtotal parathyroidectomy (4).

Results: In order to assess the results in our patients, it is useful to divide the cases into two groups. Considering only the four who had surgery without the opportunity for dialysis either preoperatively or postoperatively (Cases 1, 2, 3, and 5), three died within four months and the fourth was lost to follow-up.

With respect to the other five, two were started on chronic dialysis after subtotal parathyroidectomy for bone pain (Cases 4 and 7). Relief of symptoms was achieved and skeletal x-rays show no progression of the disease. The remaining three patients were referred from our chronic dialysis unit. Symptomatic osteodystrophy was effectively relieved in two. Severe pruritis was not alleviated in the third patient, nor was it significantly affected in two others in whom it was present preoperatively.

Medical Management: Parathyroidectomy is rarely necessary in patients who have advanced renal disease. Only three patients were referred from our chronic dialysis facilities during a period when more than 300 patients were treated.

On the other hand, about 80% of our dialysis patients have osteodystrophy which increases in severity in proportion to the length of time they have been on dialysis. Aluminum hydroxide gel (Amphogel®) has been effective in reducing serum phosphorus levels and minimizing the stimulus for bone resorption. Vitamin D and oral calcium supplements are occasionally prescribed, provided the serum phosphorus is less than 6 mg %. When it exceeds this level, the risk of metastatic calcification outweighs the potential benefit of an increased serum calcium level (5).

In a recent report, Goldsmith et al (6) suggests that a calcium level of 6 to 8 mg % in the dialysate will contribute to positive calcium balance and reduce the incidence of symptomatic osteodystrophy. In our hospital, the calcium level used in the dialysate is 5 mg % because of concern about the possibility of metastatic calcification when higher levels are used (5).

Surgical Considerations: The technique of neck exploration is identical to that described in patients with primary hyperparathyroidism. Dialysis patients were hemodialyzed the day before operation.

The amount of parathyroid tissue to be removed depends upon whether or not the patient is a candidate for renal transplantation. If so, we prefer to preserve half of a gland in anticipation of its return to normal function after a successful transplant. In those patients who are to be maintained on dialysis alone, total parathyroidectomy seems more logical. One of our patients (Case 4) clearly demonstrates the effect of a persistent stimulus for hyperplasia on the remaining half of each upper parathyroid gland. Within six years of subtotal parathyroidectomy, severe bone pain recurred, associated with a pathologic fracture. A large mass of parathyroid tissue was excised at a second operation, and this patient has done well since total parathyroidectomy was achieved.

As in patients with primary hyperparathyroidism who have severe bone demineralization, postoperative management can be difficult. Calcium replacement is usually required, and the amount may be large if bone changes are extensive. Persistent hypocalcemia directly contributed to death in one of our patients 19 days after operation.

Summary

Parathyroidectomy is seldom indicated in patients who have advanced renal disease. Our experience with nine patients suggests that symptomatic osteodystrophy unresponsive to medical management usually can be controlled by surgery. Results were disappointing in those who had pruritis. Removal of at least 3½ glands is advised. If the patient is not a candidate for renal transplantation, total parathyroidectomy may be preferable.

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Posterior Proctotomy—A Simple Approach to a Tumor in a Difficult Location

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A certain percentage of rectal tumors fall into an anatomical area that makes the decision as to type of removal a difficult one. These tumors are below the sigmoid colon peritoneal reflection. They are high enough not to be fully visualized or mobilized from the anus. To remove these, an anterior colon resection deep in the pelvis is necessary. Many times an addition of a complementary colostomy is needed. The tumors that would fall into this category are adenomatous polyps, sessile or pedunculated, with or without malignant changes, papillary adenomas, villous adenomas, and some rarer types, such as carcinoid tumors.

The adenoma that most often falls into this category is the large, broad-based villous adenoma. This tumor is a benign one, but is considered by most pathologists to be a pre-malignant type of tumor that should be totally excised. The operation that I have used for removal of this perplexing tumor is a posterior rectal colotomy and total excision. This approach has been proposed and publicized several times. Recently, a modification of this approach has been described by Mason and later by Oh.

A series of fifteen cases in the past twelve years has been personally compiled for various rectal tumors. This series includes twelve villous adenoma, two villous polypoid tumors with carcinomatous components and one carcinoid. One of these tumors with carcinomatous infiltration had an abdominal perineal resection two weeks later, and the patient is alive and well eight years after surgery. The second patient had a partially decompensated case of liver cirrhosis with ascites. Since the pathology report showed carcinomatous changes of the villous adenoma without invasion, no further surgery was done. This patient, fifteen months postsurgery is alive and shows no evidence of metastic disease. However, there has been progression of the liver cirrhosis.

The technique of this operation is simple (Figs. 1A–1C). Prior to surgery, the patient receives a bowel prep consisting of saline cathartics, saline enemata and eighteen hours of neomycin tablets by mouth. A fluid diet is given forty-eight hours prior to surgery. The patient is placed prone, and a low spinal or general anesthetic is administered. A roll is placed under the hips to elevate the buttocks. The buttocks are pulled apart with tape. The incision is made from the coccyx to the anal verge. Depending upon the configuration of the pelvis, the coccyx or the last sacral segment may be excised to give additional working room. The anococcygeal ligament is cut, and the levator ani muscles are bluntly separated

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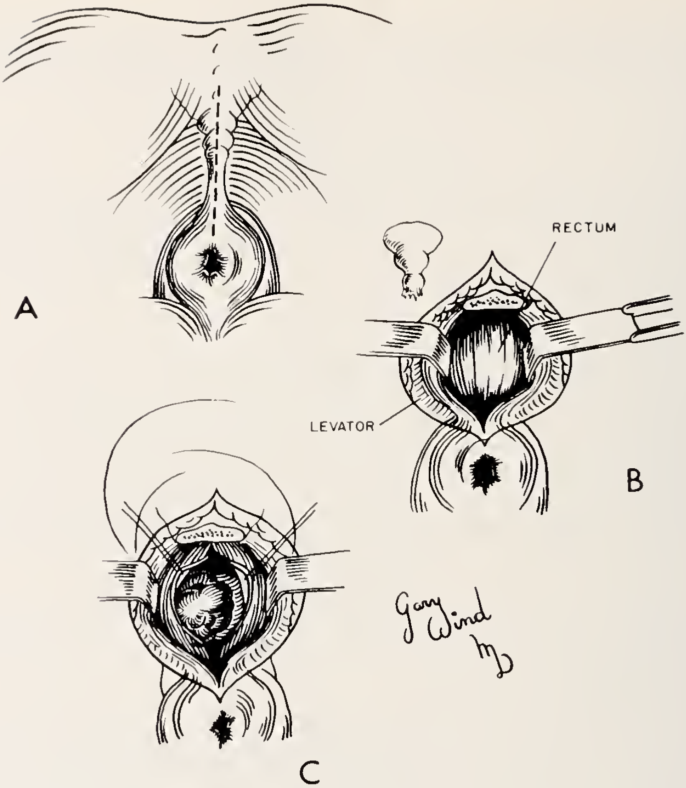

FIG. 1A. Skin incision is portrayed by dotted line showing the underlying anatomy. 1B. Coccyx and first segment of sacrum removed. Incision deepened down the midline. 1C. Immobilized rectum has been opened between stay sutures and tumor can be seen ready for removal.

from the rectum. As the incision is made in the midline, there is little bleeding, and the muscular elements are readily separated. The rectrorectal area is then entered and the rectum is remarkably mobile and easily lifted out of the space and brought up into the operative field.

Between stay sutures, the lumen is entered and the tumor is directly visualized. The tumor may be removed either with all layers or merely with the mucosa. This will depend upon the size and gross appearance. If possible, frozen sections of the questionable portions should be obtained. The repair consists of reapproximation of the mucosa as much as possible and the closure of the total defect. A two-layer repair of the opening, an inner layer of continuous Connell sutures of chromic, and an anterior layer of silk-interrupted Lembert or Cushing sutures are used. The closure may be in a line perpendicular to the inner opening, but the closure of the rectal ampulla should not give any worry as to stricture. The rectum is dropped back into the retrorectal area and two Penrose drains are brought out from each of the ischiorectal area alongside the rectum and through the main incision. After complete hemostasis, the skin and subcutaneous area are closed.

Postoperatively, the treatment is very similar to a hemorrhoidectomy. There were six temporary fecal leaks, lasting from three to twelve days. All of these closed spontaneously. There have been no major complications in this small series of cases. All cases have had sigmoidoscopic follow-up every six to eight months because the natural history of villous adenoma is for recurrence and malignant degeneration.

In my hands, total excision has proved to be far superior to multiple biopsies and fulgurations. In my hands, the Mason procedure with cutting and subsequent repair of the anal sphincters has not been necessary or advantageous. A point of controversy as to whether this posterior proctotomy and local resection could be extended to malignant tumors has been considered. Until now, this procedure has been definitive therapy for only one malignancy. More radical treatment was not deemed medically advisable in this patient. Radical cancer therapy should be bypassed only if medical contraindication would prevent a standard colon resection.

Summary

A personal series of total removal of rectal tumors high in the rectal ampulla through a posterior proctotomy is presented. This type of operative procedure is advocated as a simple but complete method of removal of certain tumors of the rectum, especially the villous adenoma.

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Leucocyte Migration Inhibition by Antisera to Immunoglobulin Chains and Fragments

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Introduction

Leucocyte migration inhibition (LMI) is an accepted in vitro test for cell-mediated immunity (1, 2). T lymphocytes are considered to be the mediators of cellular immunity and constitute approximately three-fourths of the circulating human lymphocytes (3, 4). Other in vitro tests for cell-mediated immunity include lymphocyte stimulation with increased protein and nucleic acid synthesis, and the production of transfer factor, macrophage migration inhibitory factor and lymphotoxin (3). B lymphocytes are responsible for antibody production and have abundant cell membrane immunoglobulin (Ig) determinants (4). Monomeric μ and light chain determinants have been recognized on the membranes of T cells in more and more laboratories (5–7) but the presence of Ig on T cells is not universally accepted. Hence the cells of patients with disorders of autoimmunity and increased serum Ig levels were compared with those of healthy volunteers in their response to antisera against γ, α and μ chains by LMI. LMI by antisera to ϵ, κ and λ chains, IgG Fab, IgG Fc, albumin, transferrin and by Normal Goat Serum (NGS) was also investigated, using cells of healthy volunteers. Finally, LMI by the above antisera were tested with the cells of the long term human lymphoid cell line PGLC-33H.

Material and Methods

Subjects: 1) Patients from the Department of Pediatrics of The Mount Sinai Hospital with systemic lupus erythematosus, rheumatoid arthritis and other autoimmune disorders. 2) Two female patients with IgA deficiency; one 24-year-old, with peripheral uveitis has no other past or present significant findings. The other patient aged 17 years has been asymptomatic. 3) Volunteers from The Mount Sinai Hospital personnel.

Serum γ, α and μ titers were measured on immunodiffusion plates (8) and antisera raised in goats to all chains, albumin and transferrin were obtained from Meloy Laboratories (Springfield, Virginia). Goat antiserum to human IgG Fab and IgG Fc was obtained from Miles Laboratories (Kankakee, Illinois). Control experiments were performed with normal goat serum obtained from each laboratory.

Leucocyte Migration Inhibition (LMI): Peripheral blood (30 ml) was withdrawn from patients or volunteers into plastic disposable syringes (100 U of phenol-free heparin/ml of whole blood) and allowed to sediment at 37°C for 45 minutes. The leucocyte-rich plasma was centrifuged at 90 × G for 30 minutes

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and the cells resuspended in 5 ml of complete medium (Roswell Park Memorial Institute 1640) supplemented with 20% heat-inactivated foetal calf serum, penicillin (100 U/ml) streptomycin (1 μg/ml) and fresh L-glutamine (200 μg/ml) and centrifuged at 90 × G for 5 minutes. Remaining erythrocytes, which would clump and impede migrating cells were lysed by the addition of 2 ml

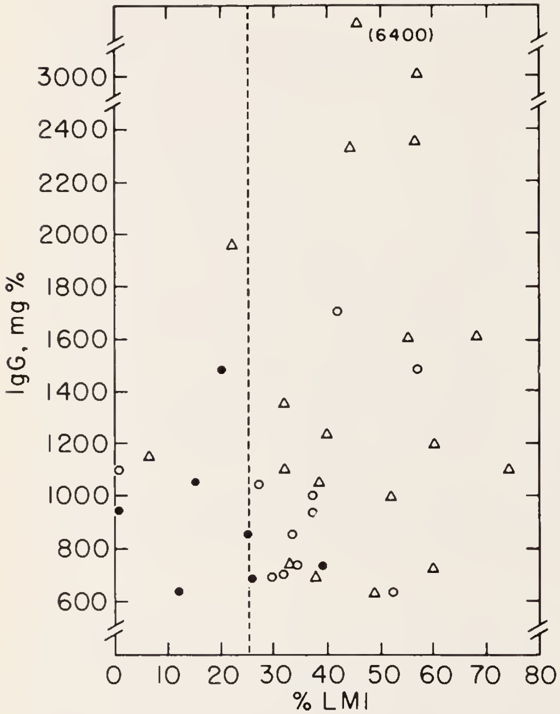

FIG. 1a

FIG. 1. Serum Ig level (mg%) and LMI by 0.2 mg antibody/ml (△) and 0.2 mg (○) and 0.1 mg (●) antiserum protein/ml raised in goats. a) Ordinate: Serum IgG, Abscissa: LMI by anti-γ; b) Ordinate: Serum IgA, Abscissa: LMI by anti-α; c) Ordinate: Serum IgM, Abscissa: LMI by anti-μ.

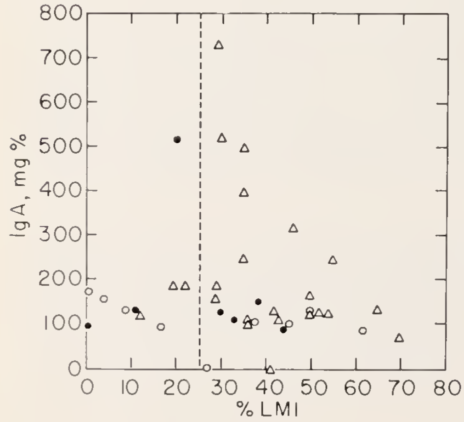

FIG. 1b

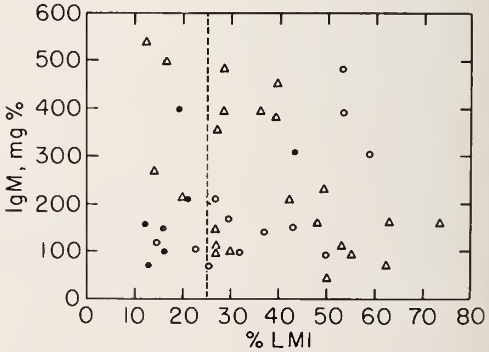

FIG. 1c

isotonic sodium chloride, followed by 6 ml of distilled water. Isotonicity was restored by the addition of 2 ml of 3.5% sodium chloride. The cells were washed twice more and a 10% cell suspension was made in complete medium. The cells were aspirated into capillary tubes (internal diameter 1.1–1.2 mm) which were sealed with plastilene clay, centrifuged at 600 × G for 10 minutes, and cut at the cell-fluid interphase. The cell-containing-portion was placed into Sykes-Moore chambers (Bellco, Vineland, New Jersey) which were filled with complete medium. At least three chambers were used for control and for each concentration of antiserum tested. The chambers were incubated at 37°C in 5% CO_2 for 18 hours. The "migration fans" were projected at fifteen-fold magnification and traced on transparent paper. The tracings were cut out and weighed on a Mettler balance. Percentage leucocyte migration inhibition (LMI) equals:

$$100 - \frac{\text{Average weight of migration fan cut-outs from chambers with antigen}}{\text{Average weight of migration fan cut-outs from chambers without antigen}} \times 100$$

25% LMI or more is considered significant in the present studies.

Experimental Procedures: Cells from patients and volunteers with a wide range of Ig levels were tested for LMI with 0.2 mg/ml of goat antibody against various Ig chains. The antisera were diluted six to tenfold in complete medium, depending on their antibody and protein concentration, for LMI studies with 0.2 mg and 0.1 mg antiserum protein/ml against γ, α and μ chains (Figs. 1a–c, 2). Volunteers cells were also tested for LMI with antiserum to ϵ, κ and λ chain, IgG Fab, IgG Fc, albumin and transferrin and with normal goat serum at a protein concentration of 0.2 mg/ml and 0.1 mg/ml (Fig. 3). Each antiserum was tested against all other antisera and chains by immunodiffusion to ascertain its purity. Precipitation occurred between anti-α and anti-μ and between anti-γ and the antisera against both light chains when protein concentration was 31 mg/ml.

Cells from the lymphoid cell line PGLC-33H were tested for LMI by the same antisera, chains and by apoferritin in the same concentrations (Fig. 4).

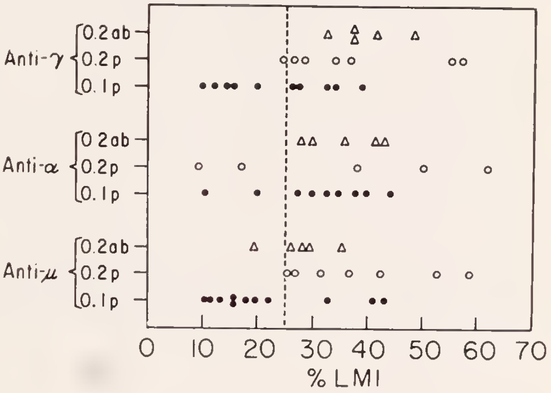

FIG. 2. LMI of cells from same individuals by 0.2 mg/ml antibody (ab) and 0.2 and 0.1 mg/ml protein (p) from antisera to heavy chains. (See Fig. 1 for symbols).

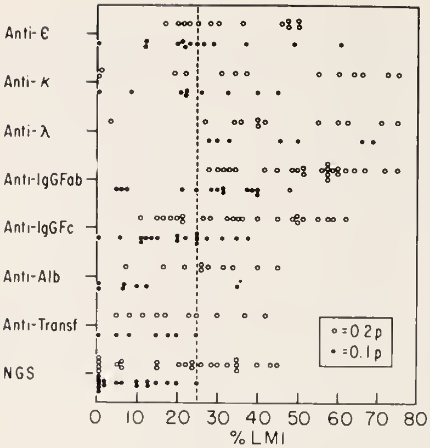

FIG. 3. LMI by 0.2 and 0.1 mg/ml protein from various antisera against immunoglobulin chains and fragments, control antisera against albumin (Alb) and transferrin (Transf) and normal goat serum (NGS). (See Figs. 1 and 2 for symbols and abbreviations).

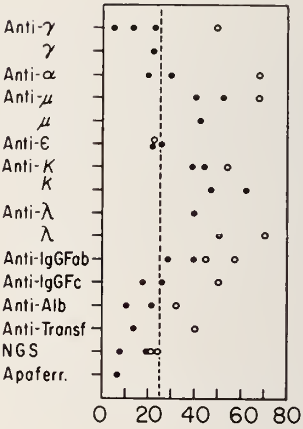

FIG. 4. LMI by 0.2 and 0.1 mg/ml protein from various antisera and 0.1 mg/ml apoferritin of cells from the lymphoid cell line PGLC-33H. (See Figs. 1 and 3 for symbols and abbreviations).

Results

Leucocyte migration inhibition (LMI) by 0.2 mg antibody/ml and by 0.2 and 0.1 mg protein/ml from antisera to γ, α and μ chain did not show any relation to the respective serum heavy chain titer. The results obtained with the cells of patients and volunteers were therefore combined (Figs. 1a–c). LMI by 0.1 mg/ml of antiserum protein did show reduced LMI, using cells of the same individuals (Fig. 2). Antisera to ε, κ and λ chains, IgG Fab, IgG Fc, albumin and transferrin and NGS generally produced LMI at a concentration of 0.2 mg/ml (Fig. 3). When the protein concentration was reduced to 0.1 mg/ml there was considerably less LMI by the diverse proteins, most noticeable with antiserum to IgG Fc but not noted with anti-λ serum (Fig. 3). Since NGS (0.1 mg protein/ml) produced 25% LMI, LMI greater than 25% at that protein concentration was considered significant for present and future studies. LMI differed by 10% or less in 11 volunteers on two or three occasions.

Comments

The present studies show no relation between the serum immunoglobulin (Ig) level to LMI by antisera to heavy chain. B cells in IgA deficiency have been shown to have IgA membrane determinants (9); the two patients with IgA deficiency showed LMI with anti-α serum. The use of radioimmunoassay might detect low levels of circulating IgA in these patients. IgE was discovered by radioimmunoassay which is more sensitive than immunodiffusion (8, 10).

The present results suggest that several interactions may lead to LMI. If T cells are the primary cells involved, stimulation must occur via an as yet undetected Ig membrane determinant. The cells may then secrete migration inhibitory factor (MIF) and/or produce transfer factor leading other cells to secrete MIF. B cells may be stimulated to produce MIF by an antigen/antibody interaction at the cell membrane with pinocytosis of the complex. Another explanation may be that the antigen/antibody complex accumulates more and more Ig molecules so that the cell bearing the aggregate will impede migration. This phenomenon has been shown to occur with guinea pig macrophages and antigen/antibody/complement complexes (11). The production of LMI with antiserum to α chain makes it more likely that the B cell is the principal cell involved. In addition, long-term human lymphoid cell lines, presumed to be B cells, have been shown to elaborate MIF with a MW between 12,500 and 30,000 (12).

A cell membrane antigen/antibody complex can impede migration by involving leucocytes other than lymphocytes. Receptors for IgG have been described on human monocytes and for migration inhibitory factor on peritoneal guinea pig macrophages (13, 14). The migration of PMN's in the LMI test may be influenced by their IgG membrane determinants (15, 16). Phagocytosis of aggregated gamma globulin and immune complexes, and the resulting enzyme release as well as their own chemotactic action probably play a role in the test (15, 17). Macrophage-bound antigen plus T and B cell interaction may enhance LMI.

The PGLC-33H lymphoid cell line originally produced γ, α, μ, κ, and λ chains (18). The cells recently studied by radioimmuondiffusion and immunofluorescence were shown to carry only μ and κ chain membrane receptors (19). μ and κ were the only Ig molecules secreted but cell lysates contained 8 γ for every 53 μ molecules and 33 λ for every 142 κ chains (20). Protein 0.1 mg/ml from antiserum to μ, κ and λ chains and to IgG Fab produced significant LMI but not from antiserum to IgG Fc and γ chain. Doubling the protein concentration of antisera or Ig chains caused LMI in additional instances (Fig. 4). In the case of λ chain no membrane determinant explains this result. Anti-λ serum caused other discrepancies (Fig. 3).

Further studies regarding the above findings are in progress. Human leucocyte migration inhibition (LMI) has been studied as an in vitro test for cell-mediated immunity in disorders of the gastrointestinal tract, liver, kidney, eye, central nervous system and of autoimmunity (21–24). The present experiments suggest that the evaluation of cellular immunity by LMI remains equivocal.

Summary

The serum IgG, IgA and IgM titer is not related to leucocyte migration inhibition (LMI) by antisera to γ, α and μ chains. LMI of cells from the long-term lymphoid cell line PGLC-33H by antisera against Ig chains and fragments was evaluated. LMI appears due to migration impairment by cell bound antigen-antibody complexes when the antigen is a membrane determinant; it may involve cells other than T lymphocytes. The amount of protein used as antigen influences LMI. Care must be taken before immunologic significance is attributed to the results.

Acknowledgments

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The Relationship Of Urinary Androgen Metabolites to Breast Diseases Including Carcinoma

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Accumulating data suggest a close relationship between the amounts of androgen and corticosteroid metabolites excreted in the urine and the clinical course of breast cancer. Reports claim further that abnormalities in excreting these compounds may be associated with a high risk of subsequent breast cancer in ostensibly normal women. Bulbrook (1), Stern (2), and others questioned whether these abnormalities were a consequence of the tumor or whether they preceded the clinical appearance of disease. Marmoston (3) states that postmenopausal patients with breast cancer excrete significantly higher levels of total estrogen than patients without malignancy. Bulbrook (4) found that some patients with breast cancer at an early stage of the disease excreted subnormal amounts of 11-desoxy-17-ketosteroids. Allen (5), on the other hand, reported urinary 17-hydroxycorticosteroids to be normal in most patients with advanced breast cancer and noted no difference in urinary 17-hydroxycorticosteroids between patients responsive and nonresponsive to therapy.

Approximately two years ago, the Breast Service of Elmhurst General Hospital in cooperation with the Clinical Pathology Department decided to conduct its own survey of this intriguing problem to determine whether or not there was any definite pattern of the urinary excretion of these hormones which could be correlated with the clinical status of the patient.

Method

The patients studied were all female and were divided into four categories:

A. Normal Controls
B. Benign Breast Disease
C. Untreated Breast Cancer
D. Mastectomized Breast Cancer

Each category was subdivided into premenopausal and postmenopausal. In all, there were 163 women studied (Fig. 1). The *first category* consisted of women who appeared at the OPD clinic for complaints referable to the breast but had no clinical evidence of pathology. The *second group* were women who had obvious benign lesions such as cysts, fibroadenomas, fibrosis, etc. The *third group* had a proved breast carcinoma without metastasis and had not received any therapy, and the *fourth group* were postoperative patients who had had

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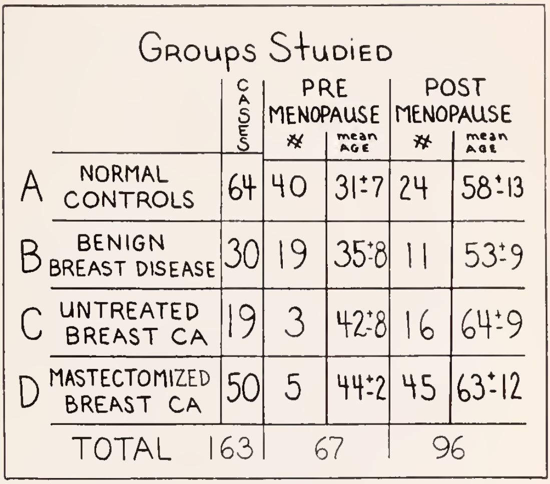

Groups Studied

		CASES	PRE MENOPAUSE		POST MENOPAUSE	
			#	mean AGE	#	mean AGE
A	NORMAL CONTROLS	64	40	31±7	24	58±13
B	BENIGN BREAST DISEASE	30	19	35±8	11	53±9
C	UNTREATED BREAST CA	19	3	42±8	16	64±9
D	MASTECTOMIZED BREAST CA	50	5	44±2	45	63±12
	TOTAL	163	67		96	

FIG. 1. Age and numbers of patients studied.

their carcinomas removed and had no demonstrable distant metastasis at the time of their urine studies.

Each female studied submitted a 24-hour urine specimen. Urine creatinines were determined to monitor the completeness of urine collection. Aliquots consisting of about 300 ml of urine were stored frozen until analyzed. Total 17-KS were determined by the method of Drekter et al (6). The total 17-OHCS were determined by the ketogenic method of Few (7) using DHIA as the standard. The etiocholanolone and the combined DHIA and androsterone were determined using the TLC system of Miller and Durant (8). The latter was modified to use the combined glucorondiase-sulfatase hydrolysis step as recommended by Holub (9).

Data

Figure 2 presents bar graphs summarizing the average 24 hour 17-KS urinary secretion observed in the four patient group. No differences could be found. There were also no differences in total 17-OCHS. Figure 3 illustrates the findings with regard to the etiocholanolone levels. Comparing the postmenopausal control 24-hour urinary etiocholanolone excretion with the levels observed in both the treated and untreated postmenopausal breast cancer patients indicated a decreased urinary excretion in the latter which was statistically significant at the 5% level. No differences could be seen in the premenopausal groups. The combined DHIA-androsterone levels are demonstrated in Fig. 4. Comparison of the postmenopausal control group to both the postmenopausal cancer groups also indicated a statistically significant difference at the 5% level with the cancer patients excreting less than the noncancer controls. This trend of subnormal steroid excretion in cancer patients is even more evident in comparisons of 24-hour levels of 11-desoxysteroids (Fig. 5). The data indicating de-

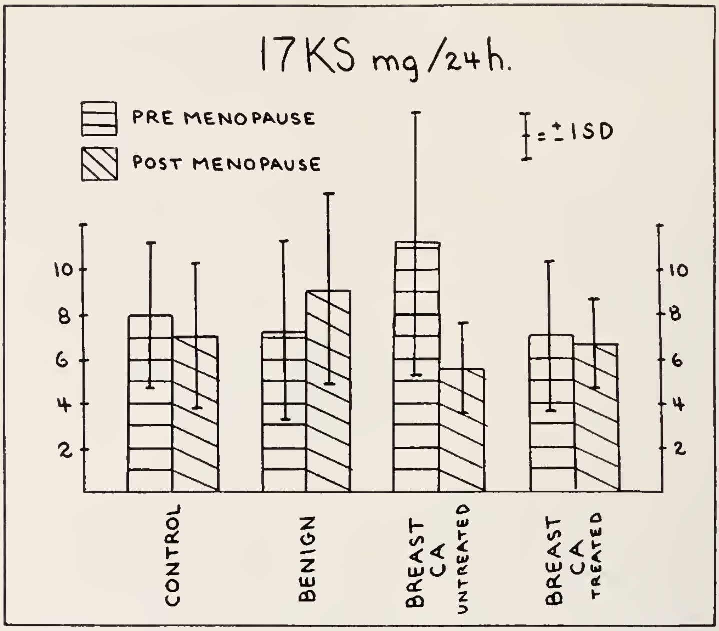

FIG. 2. Average 24-hour urinary 17-KS excretion in mg among the four study groups.

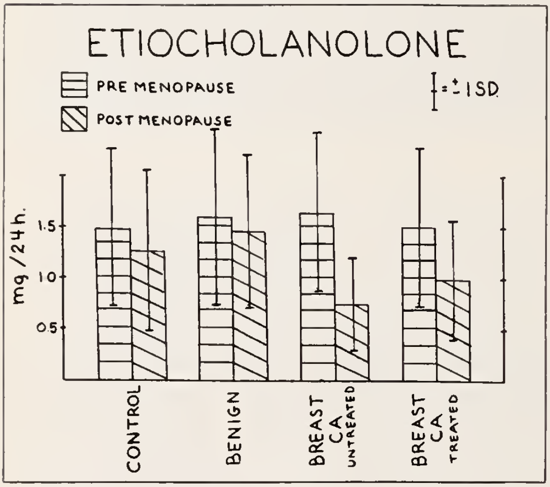

FIG. 3. Average 24-hour urinary etiocholanolone excretion of the four study groups.

creased secretion in the postmenopausal patients compared with noncancer postmenopausal controls was significant at the 1% and 0.5% levels.

Comparison of the postmenopausal noncancer group to the postmenopausal cancer group in Figure 5 has a significant value at the 1% level for etiocholanolone and at .1% for the DHIA-androsterone level.

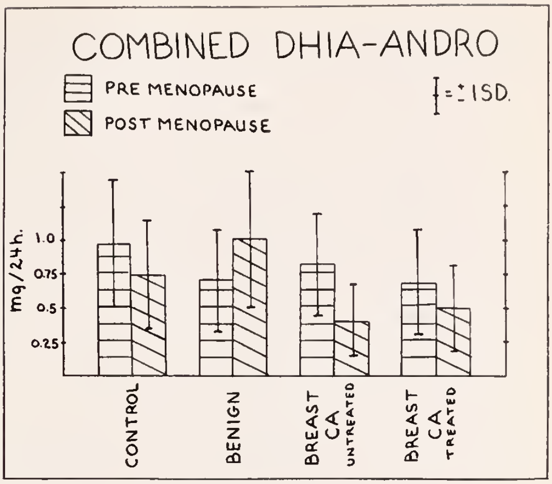

Fig. 4. Combined average DHIA-androsterone urinary excretion levels observed in the four study groups.

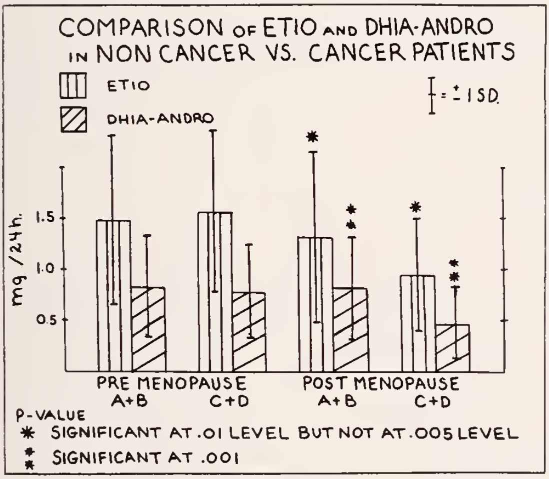

Fig. 5. Comparison of ET10 and DHIA-ANDRO in noncancer versus cancer patients.

Discussion

The urines of 163 women were examined in the Breast Clinic of Elmhurst General Hospital for androgen metabolites. They were divded into groups according to the presence of breast pathology and their menstrual status. No difference could be found in total 17-KS or 17-OHCS between the groups studied. However important differences were found in the levels etiocholanolone and DHIA-androsterone in postmenopausal breast cancer patients when compared to noncancer subjects. This significant difference could be applied as a mass screening test for all postmenopausal females. Patients with positive urinary results can be separated for further clinical evaluation as to the presence of breast carcinoma.

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The Systematic Preoperative Evaluation of Eustachian Tubal Function

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Ventilation of the middle ear cleft is essential for successful tympanoplastic surgery. In patients with tympanic membrane perforations, the preoperative evaluation of eustachian tubal function may be useful in identifying those subjects with nonfunctioning or blocked eustachian tubes. Additionally, the anatomic localization and identification of the structural abnormalities affecting tubal function may permit specific corrective therapy to be undertaken.

The proposed approach for clinically evaluating eustachian tubal function combines the gross micro-otoscopic examination of the middle ear mucosa, the evaluation of the eustachian tube's ability to equilibrate induced middle ear pressures and, in selected cases, the radiographic visualization of the eustachian tube.

Using these parameters, a systematic approach has been designed which is useful in preoperatively evaluating the eustachian tube in patients with tympanic membrane perforations who are being considered for middle ear reconstructive surgery.

Clinical Methods

1. *Examination of Middle Ear Mucosa*

Patients presenting with tympanic membrane perforations are examined using a standard operating microscope. Attention is focused on the mucosa as visualized through the tympanic membrane perforation. Its appearance is subjectively appraised. The normal lining is a thin, translucent, shiny, refractive mucoepithelial membrane with distinct blood vessels and is exemplified by the middle ear mucosa as observed during stapes surgery. In patients with dry chronic tympanic membrane perforations, the appearance of the mucosa may be altered. Characteristically it is thicker, more opaque, does not reflect light as well, and has a pinkish color and velvety texture. The small promontory vessels lose their distinct identity. The mucosal surface, when obviously abnormal, will have a dull, edematous, heaped-up appearance. If the mucosal surface is markedly thickened, the lining appears polypoid, obliterating the middle ear cleft. When acutely infected, the mucosa will have an erythematous inflamed appearance and a muco-purulent discharge will be evident. In chronic cases, the mucosa will have a dull, boggy, vasomotor-like appearance and mucoid otorrhea may be noted.

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2. *Insufflation-Aspiration Test of Eustachian Tubal Function*

This test evaluates the ability of a patient with a tympanic membrane perforation to equilibrate an induced middle ear pressure. The Madsen ZO-70 electro-acoustic impedance bridge, originally designed for tympanometry, can be used for performing this test of eustachian tubal function (Fig. 1). This apparatus incorporates a manometer with the capability of varying the air pressure within a closed cavity. The instrument probe is inserted into the external ear canal and an airtight seal is established. The air pressure within the sealed middle ear-external canal system is varied. Initially, a positive pressure of 400 mm of H_2O is insufflated into the middle ear. The patient is given water and asked to swallow repeatedly. The point at which the pressure in the middle ear equilibrates is recorded. Next, a negative pressure of -200 mm H_2O is created and the process is repeated. The resulting residual middle ear negative pressure is recorded. This examination is simple to perform and takes less than 5 min. It can be done by a trained nurse, technician, or audiologist. Using the above technique, patients have been categorized into three separate groups. Group I consists of patients having adequate eustachian tubal function whereby positive insufflation will immediately pass through the tube while a negative pressure will be equilibrated to within -100 mm of H_2O. Group II is composed of patients with patent, though abnormally functioning, eustachian tubes. In this group, a positive pressure will be noted to be equilibrated whereas a residual negative pressure of -100 mm of H_2O or greater will persist. Group III contains patients with blocked eustachian tubes with the result that neither a positive or negative middle ear pressure will be equilibrated.

3. *Radiographic Examination of the Eustachian Tube*

Those patients noted to have blockage of the eustachian tube (Group III) are examined radiographically. In order to visualize the eustachian tube fifty percent sodium diatrozoate USP* is instilled into the middle ear through the tympanic membrane perforation (Fig. 2). Using image intensification radiographic techniques, the middle ear-eustachian tube area can be outlined. Fluoroscopically, the progress of the dye can be observed as the patient swallows and performs the Valsalva and the Toynbee maneuvers. In patients with nasopharyngeal disease, the cartilaginous portion of the eustachian tube can be visualized by retrograde filling from the nasopharynx. By utilizing these techniques, anatomical areas of obstruction existing with the middle ear-eustachian tube complex can be identified.

Results (Tables IA, IB)

Thirty-eight patients with tympanic membrane perforations were evaluated. Eustachian tubal tests were performed after attempts had been made to control active infection.

Seven of these patients had good eustachian tubal function (Group I). The

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* Hypaque Sodium 50%® (Winthrop).

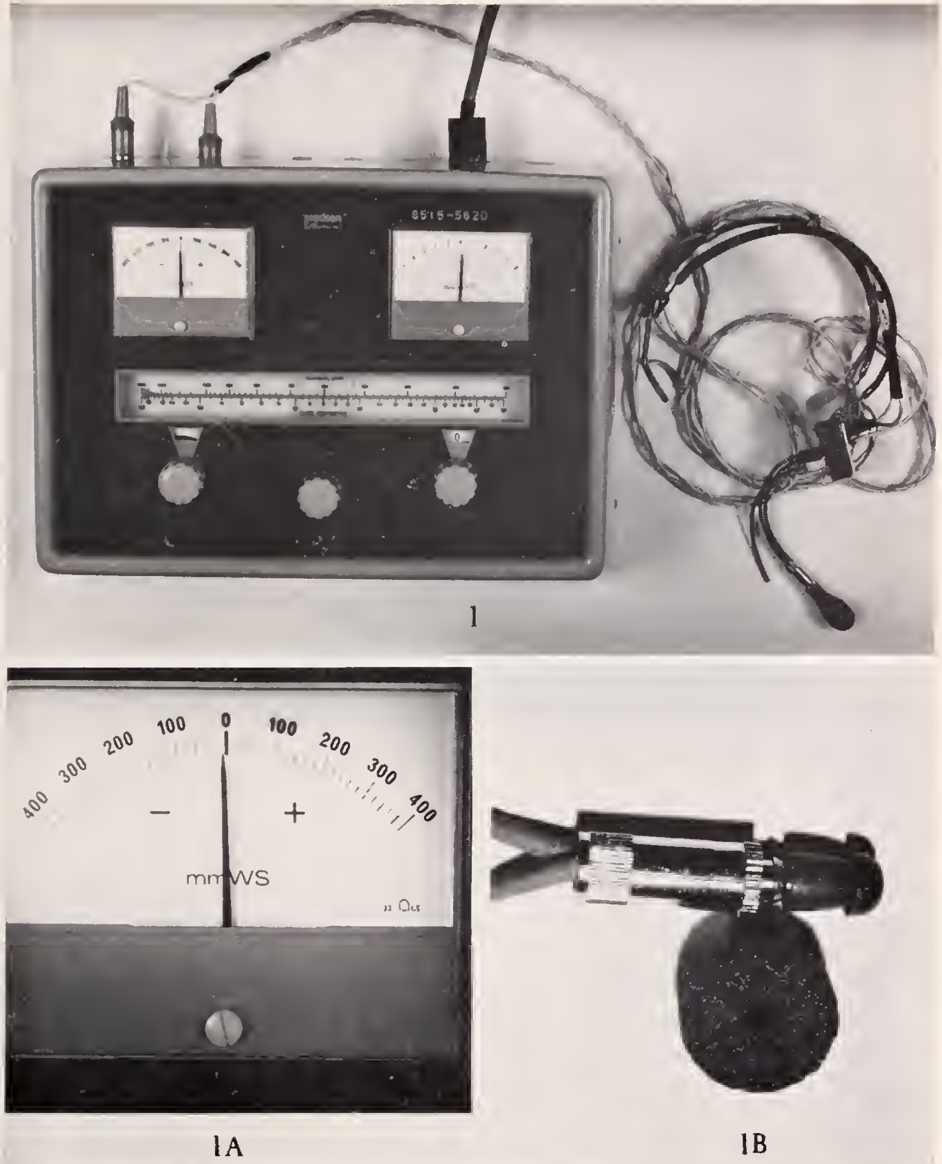

Fig. 1. Madsen ZO-70 electro acoustic impedance meter used in performing the insufflation-aspiration eustachian tubal function test. 1A: Manometer meter indicating induced pressure. 1B: Ear insert and sealing tips which permits an air tight seal of external canal.

mucosa was felt to be normal in three of these seven patients. The other four had slightly thickened dull middle ear mucosa, which was not markedly abnormal; none had otorrhea.

Twenty-five patients were observed to have moderately impaired eustachian

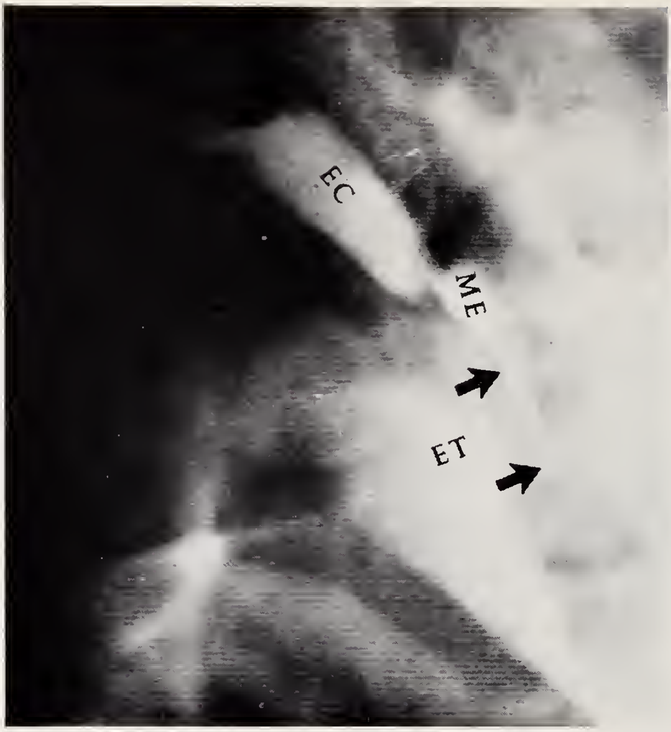

Fig. 2. Radiographic contrast study of normal eustachian tube. EC: external canal. ME: middle ear. ET: eustachian tube.

TABLE IA

Relationship of Mucosal Abnormalities to Eustachian Tubal Function (38 Ears)

Eustachian Tubal Function	No. of Ears	Middle Ear Mucosa			Otorrhea
		Normal	Thickened	Abnormal	
Group I	7	3	4		
Group II	25	3	20	2	2
Group III	6	0	0	6	4

TABLE IB

Radiographic Evaluation of Blocked Eustachian Tube (Group III)

6 Patients	
Apparent Blockage	
Retrograde Passage of Air (Valsalva Maneuver)	4
Anatomical Blockage	
Localized to Tubo Tympanic Recess	2

tubal function (Group II). Twenty of these patients were felt to have slight mucosal thickening. In two patients the mucosal abnormality was more severe and a persistent slight mucoid otorrhea was present. The remaining three patients were felt to have normal mucosa.

Six patients were noted to have blocked eustachian tubes (Group III). All six were observed to have abnormal mucosal changes. Four of the six patients had active otorrhea. One patient had persistent mucopurulent discharge with an actively inflamed polypoid mucosal surface, visible through the tympanic membrane perforation.

The six patients in Group III were examined by means of eustachian tubal contrast radiography. Using this technique, in four of these patients retrograde passage of air from the nasopharynx could be observed. During the Valsalva maneuver, air bubbles could be seen passing through the contrast media that had been retained within the middle ear. In the two patients in whom an anatomical area of obstruction was demonstrated, the site of blockage was localized to the tympanic portion of the eustachian tube and was attributed to the abnormal mucosa.

Discussion

The success of myringoplastic surgery is measured by the success of grafting techniques and by the postoperative hearing level that is achieved. The creation of an effective sound transmission mechanism requires that an aerated tympanic cleft be maintained postoperatively. In turn, the establishment of a stable middle ear space is dependent upon the presence of a mucosal lining and of an adequately functioning eustachian tube.

Three recent developments have proven most useful in evaluating the status of the eustachian tube. First, the mucosal surface of the middle ear has been carefully studied using both histologic and electron microscopic techniques. The complex morphologic nature of this mucociliary system and its response to pathologic processes have been described (1–10). These investigative studies have indicated the importance of examining the middle ear epithelial lining in patients in whom eustachian tubal function is being evaluated. Secondly, physiologic tests designed to determine the eustachian tube's functional status in people with tympanic (11–17) membrane perforations have been described. By evaluating the ability of these patients to equilibrate and induced middle ear pressure, subjects with compromised tubal function can be identified. Finally, sophisticated radiographic equipment has been utilized to fluoroscopically visualize the eustachian tube in order to further evaluate eustachian tubal function and to localize anatomical areas of obstruction (18–21).

Eustachian tubal function tests have demonstrated that the majority of patients with tympanic membrane perforations have abnormal function (Table II). However, the results of tympanic membrane reconstruction in several large series have been generally good, with reported success rates of 86–99% (22–25). Several investigators have correlated the functional status of the eustachian

TABLE II

Incidence of Abnormal Eustachian Tubal Function Observed in Patients with Tympanic Membrane Performance

Author	Reference	Number of Ears Tested	Percent of Ears Tested Having Abnormal Function
Miller, G.	12	180	100%
Siedentop, K. H. et al.	13	244	86%
Holmquist, J.	14	217	78%
Flisberg, K.	15	102	58%
MacKinnon, D. M.	16	80	54%
Ekvall, L.	17	39	54%

TABLE III

Success Rate of Myringoplastic Surgery Correlated with Functional Status of the Eustachian Tube

Author	Reference	No. of Ears	Parameters of Successful Surgery	Normal E.T. Function	Surgical Success Rate	
					Moderately Functioning E.T.	Poorly Functioning E.T.
MacKinnon, D. M.	16	80	Graft Take	94%	100%	73%
			Hearing Result	81%	80%	42%
Holmquist, J.	26	89	Graft Take	75%	35%	22%
Siedentop, K. H. et al	27	30	Graft Take	82%	75%	60%
Palva, A. et al	28	25	Graft Take	100%		87%
Miller, G. et al	29	22	Graft Take (Hearing Result M.E. Aeration)	88%	33%	0%

TABLE IV

Correlation of Eustachian Tubal Radiographic Clearance Test with Results of Myringoplasty

Status of Eustachian Tube	No. of Ears	No. of Failures	Success Rate
Nonfunctioning	6	3	50%
Functioning	19	3	84%

tube with the surgical success rate of myringoplastic surgery (Table III). In general, those patients with normal or moderately functioning patent eustachian tubes had good results, while the surgical results in patients with poorly functioning, or obstructed tubes, were generally poor. Similar observations were made in 24 patients with tympanic membrane perforations whose eustachian tubal function were evaluated using a radiographic clearance technique (20, 21). In patients noted to have normal clearance, an 84% success graft rate was attained, while in those patients in whom clearance was abnormal, the surgical success rate diminished to 50% (Table IV).

From these observations it becomes evident that although many or all patients with tympanic membrane perforations will have abnormal eustachian tubal function, most would be expected to do well surgically. Only patients with severely compromised tubal function or tubal blockage present a problem.

Functional studies of the eustachian tube can be useful in selecting patients with nonfunctioning or blocked eustachian tubes. However, these tests alone do not help to localize the responsible pathologic processes. The aim of the described systematic evaluation of eustachian tubal function is to demonstrate causative structural abnormalities in patients who have been noted to have poorly functioning or obstructed tubes.

In recent years, histologic (1, 3) and electronmicroscopic (2, 8, 10) studies of the middle ear have demonstrated that the mucosa consists of a ciliated columnar epithelium containing secretory cells. This lining is similar to and continuous with the mucosal lining of the eustachian tube. Although abnormal middle ear mucosa is often observed in association with a poorly functioning eustachian tube (30–32), tubal function may occasionally be normal even in the presence of mucosal disease. Pathologic states of the upper respiratory tract can affect the eustachian tubal mucosa thus disturbing the eustachian function and extending to secondarily involve the middle ear. Alternatively, localized middle ear pathology can block the tympanic orifice of the eustachian tube. In these patients, even though no abnormalities exist intrinsically within the eustachian tube, nevertheless, function studies would be abnormal. Therefore, it is evident that identification of middle ear mucosal abnormalities, as visualized through the operating microscope, will help the clinician interpret the results of eustachian tubal function tests, and, in certain cases, may localize the cause of the altered function.

The radiographic visualization of the eustachian tube using a radio-opaque contrast material is recommended in patients in whom eustachian tubal obstruction has been diagnosed. It will serve to confirm the presence of a blocked eustachian tube and may be of value in localizing the site of obstruction. It has been noted that in some patients in Group III in whom a positive pressure could not be insufflated from the middle ear through the eustachian tube, retrograde passage of air from the nasopharynx into the middle ear could be observed radiographically as these patients performed the Valsalva maneuver. This procedure demonstrated that their eustachian tubes were patent. In the two patients in whom the tube was noted to be blocked, the obstruction was localized to the tympanic portion of the eustachian tube. At surgery, the tubotympanic recess was noted to be occluded by thickened abnormal appearing mucosa which was removed. The denuded area was then lined by 0.05 mm teflon-reinforced silastic sheeting, and the perforation was grafted using temporalis fascia. Postoperatively, both patients were noted to have intact tympanic membranes and an aerated middle ear cleft.

By utilizing this systematic multifaceted approach for evaluating eustachian tubal function, problem patients can be identified, and the factors causing poor tubal function or tubal obstruction can be localized, allowing for more effective therapeutic management. Frequently, the problem may be localized to the tympanic portion of the eustachian tube, and attempts to correct the defects can be made at the time of tympanoplastic surgery. When considering the inconvenience of performing these tests of eustachian tubal function, it should be considered that in patients with compromised tubal function, unless means of aerating the middle ear space can be established, the chances of achieving a good hearing result may be jeopardized even if closure of the tympanic membrane is achieved.

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Factitious Uric Acid Urolithiasis as a Feature of the Munchausen Syndrome

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Abstract

Factitious disease is generally not considered in the differential diagnosis of urolithiasis. The present report describes self-induced urolithiasis in a young registered nurse. Diagnosis was established after more than one year of disease and intensive work-up and observation in a Clinical Research Center.

The diagnosis of self-induced disease is often difficult. In the patient reported here, diagnosis was delayed because of the unusual clinical manifestations of her factitious disease.

Case Report

The patient, a 21-year-old white registered nurse, was admitted to the Clinical Research Center (March, 1973) for evaluation of recurrent uric acid urolithiasis.

One year before admission she reported the onset of recurrent acute right abdominal pain accompanied by nausea and vomiting. One month later an exploratory laparotomy and appendectomy were performed, but no abnormalities were noted. The episodes of pain became severe and occurred once weekly. In June, 1972 she consulted a urologist and reported having passed a large renal stone. Cystoscopy was performed and a single stone (1 × 1 cm) was extracted from the bladder. The stone was found to be 100% uric acid. Between June and November of 1972 she had repeated bouts of right flank pain and hematuria and underwent multiple cystoscopies with extraction of more than 40 stones from her bladder. Two intravenous pyelography studies, done in December 1972 and February 1973, were normal. Serum uric acid on multiple determinations were generally in the normal range. Urinary uric acid was reported to be 1000 and 1400 mg/24 hours. Treatment with allopurinol resulted in a decrease in serum uric acid to less than 4.0 mg%, but she continued to report renal colic and uric acid stones were removed from her bladder at a frequency of one per week. The dose of allopurinol was increased to 1 gram/day along with 12 grams/day of sodium bicarbonate, but she continued to report renal colic and uric acid stones were removed from her bladder at the same frequency. She was hospitalized in the Clinical Research Center for further evaluation.

Social history revealed that the patient was the third of four children in the family. There was no history of gout or urinary stones in the family and close relatives. Elderly grandfather has suffered from chronic arthritis and mother had diabetes.

Physical examination disclosed no abnormalities other than marked obesity (205 lbs.); blood pressure of 120/80 mm; urinalysis revealed specific gravity of 1.020; pH of 6.0; no glucose or protein, and 2–3 white cells per high power field. The hematocrit was 40.0 percent, hemoglobin 12.5 g; the white blood cell count was 9100. The urea nitrogen was 11 mg; the creatinine 1.0 mg. Fasting plasma glucose was 90 mg%. A 6-hour oral glucose tolerance test was normal. Total serum protein was 6.5 g (albumin 3.8 g) per 100 ml. Serum sodium was 142 mEq, potassium 4.3 mEq, chloride 105 mEq and carbon dioxide 28 mEq per liter. She had normal bilirubin, SGOT, and alkaline phosphatase.

Clinical Research Center Studies

The stones, multiple samples of which were carried by the patient (Figure 1), were found to be 95% uric acid (uricase method) by weight. Serum uric acid ranged between 5.4–5.8

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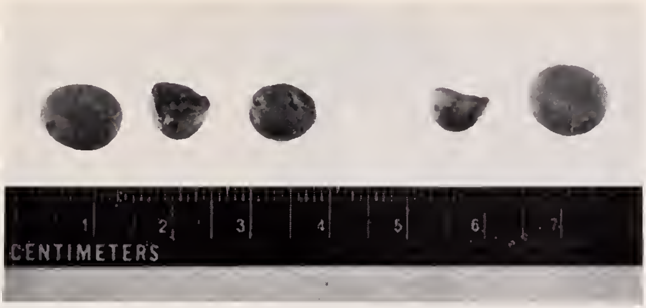

Fig. 1. Stones removed by cystoscopy.

mg% on no therapy and urinary excretion of uric acid after 5 days on a free-purine diet ranged from 252–474 mg/24 hours. Assay for hypoxanthine-guanine-phosphoribosyltransferase revealed normal activity.

Hospital Course

On the third hospital day, the patient began complaining of intermittent colicky back pain radiating to the right lower quadrant of her abdomen with urinary frequency and nausea. Symptomatic treatment with pentazocine and diazepam was started. On the fifth day, the pain was localized to the lower abdomen and she was tender to palpation over the bladder. Urine culture revealed growth of E. coli. The patient stated that she could feel the stone pressing down her urethra to the bladder and claimed that heavy pressure applied to the right side of abdomen hastened the travel of the stone. On the fifth hospital day, she indicated that she felt the stone pop into the bladder; after this the pain abated. Thereafter she was unable to void spontaneously and urethral catheterization was required to empty her bladder. On the eighth day, she had cystoscopy. Two stones were extracted at cystoscopy and given to the patient. She was asymptomatic until the eleventh day of hospitalization, when colicky back pain recurred. On the eighteenth hospital day, two additional stones were removed by cystoscopy. She again was asymptomatic until the twenty-third day, when pain recurred with persistent nausea. An intravenous pyelogram was done during an episode of colicky pain. The study showed a single stone within the bladder, kidneys and ureters were normal (Figures 2, 3). Although the patient stated she had

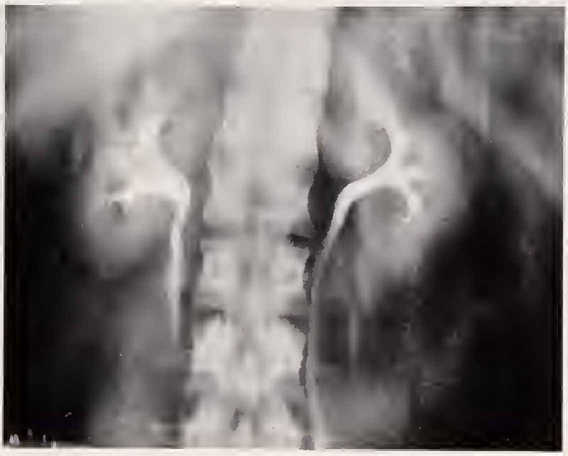

Fig. 2. Normal kidneys and ureters at the time of renal colic.

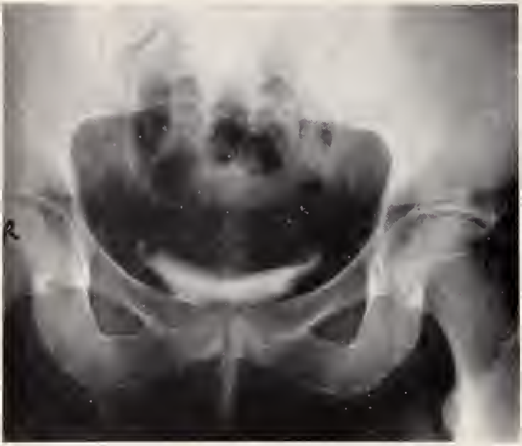

FIG. 3. A single stone is seen in the bladder.

turned over all the stones in her possession to her physician, additional stones were found in her room. The source of the uric acid stones in her possessions was unknown. The patient had been insisting that the stones removed from her bladder be turned back to her after every cystoscopy. She also concealed 20 mL of clotted blood in her room. These tubes disappeared on the same night that the patient had an episode of "hematemesis."

Psychiatric consultations were sought. The psychiatrist described her as an anxious, average, intelligent patient with no evidence of hallucinations or delusions. He believed that her symptoms were factitious and that the illness served as an expression of otherwise repressed needs for exhibitionism and sexual gratification. She enjoyed the tremendous attention focused on her by the doctors, medical students, and staff. His recommendations were to confront the patient with the truth about her disease, to explain to her its harmful effects on her body, and to advise her to seek further psychiatric help.

In view of this recommendation, the patient was informed that we could not find any cause for her illness and that we had suspected that the disease was self-induced. Although she admitted to concealing uric acid stones in her room and simulating upper gastrointestinal hemorrhage, using old blood, she never admitted to inserting stones into her bladder.

After receiving this information, the patient left the hospital and refused psychiatric follow-up. Her previous physicians were contacted and informed of our findings.

Comments

The dramatic features of this illness were recurrent episodes of renal colic followed by appearance of uric acid stones in the bladder. Features that suggested factitious disease include: 1) The unusual number of stones passed; 2) The normal appearance of the kidney and ureters on multiple intravenous pyelogram studies; 3) The absence of metabolic abnormality in purine metabolism, and the presence of normal urinary uric acid and urine pH; and 4) The constant desire of the patient for urethral and bladder manipulation. The correct diagnosis was made after evaluation in the Clinical Research Center and was indicated by laboratory tests that were incompatible with her clinical manifestations. The diagnosis was confirmed by psychiatric evaluation followed by direct confrontation with the patient.

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RADIOLOGIC NOTES

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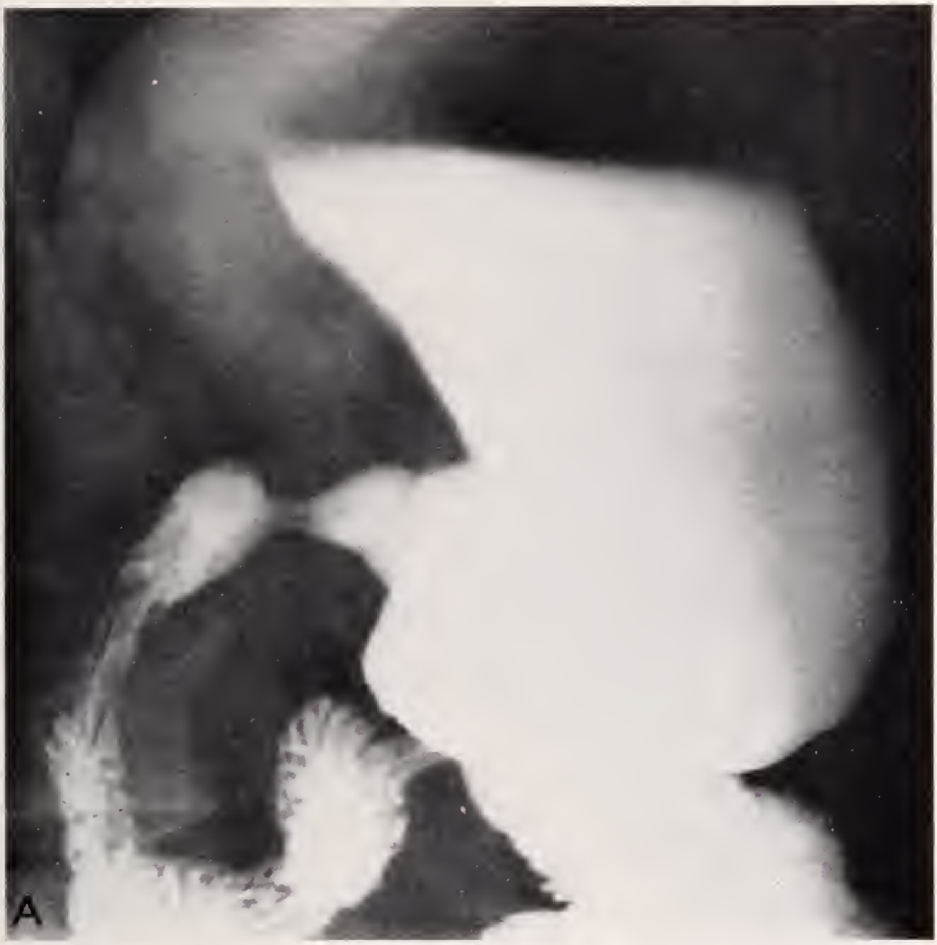
Case No. 383

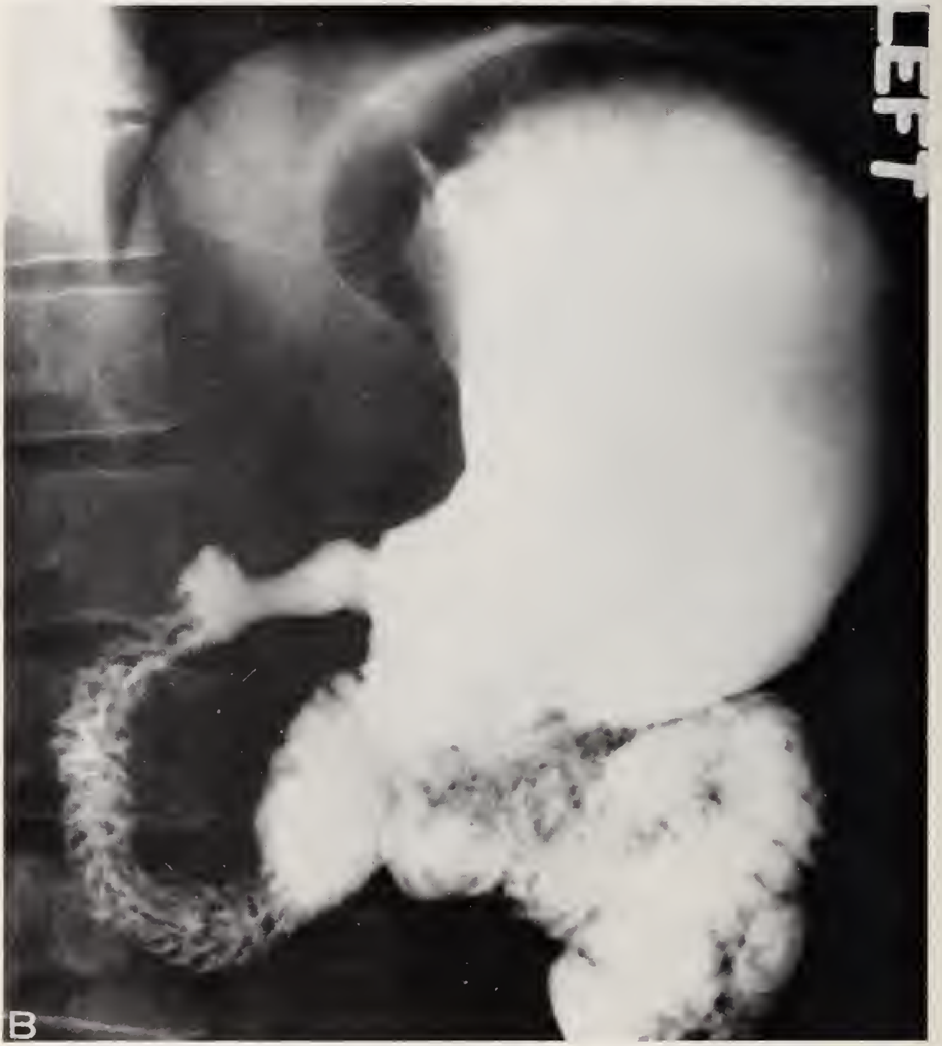
This 56-year-old man was diagnosed as having Crohn's Disease of the small bowel at the age of 27, while serving in the Army. At that time, he noted symp-



Case 383, Fig. 1. A, Examination of the stomach in the erect right anterior oblique view reveals a concentric narrowing of the antrum of the stomach with a patulous pylorus and a deformed duodenal bulb. The duodenal sweep is normal. No gastric ulcerations or mass lesions are noted. B, In the lying-down right anterior oblique view, there is also some foreshortening of the lesser curvature of the stomach in its distal half. C, Spot film in the left posterior oblique view, with air in the distal portion of the stomach confirms the conical narrowing of the distal portion of the stomach.

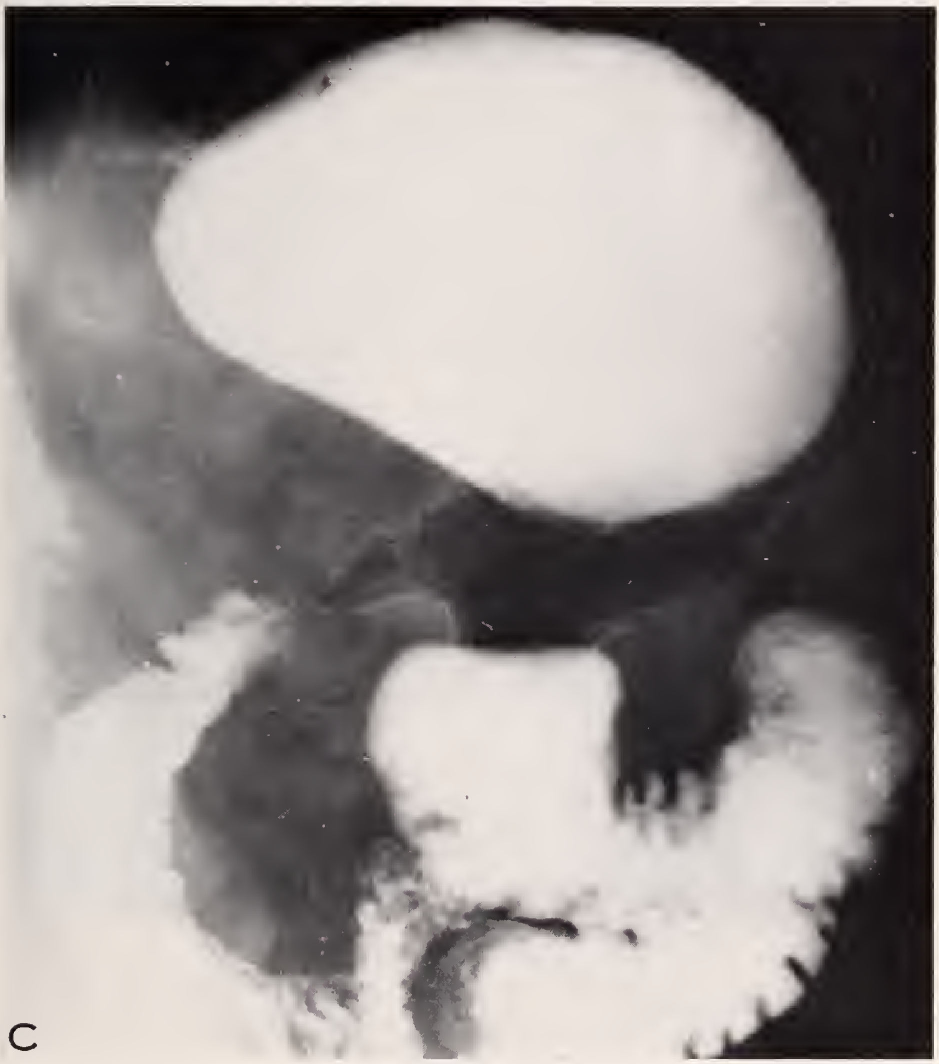
toms of diarrhea, occasional vomiting and weight loss, together with intermittent fever. He was treated conservatively and then experienced a long interval without gastrointestinal symptoms. At the age of 47, he had the onset of mild diarrhea, again responding well to supportive measures. At the age of 50, he presented with marked fatigue, which was related to a profound anemia that responded rapidly to iron and vitamin B_{12} therapy. The patient had very few gastrointestinal symptoms, but experienced occasional bouts of low grade fever. He noted no vomiting, diarrhea, arthralgias or urinary symptoms.

Radiographic examination of the upper gastrointestinal tract showed a marked concentric narrowing of the distal third of the stomach without mass lesions or



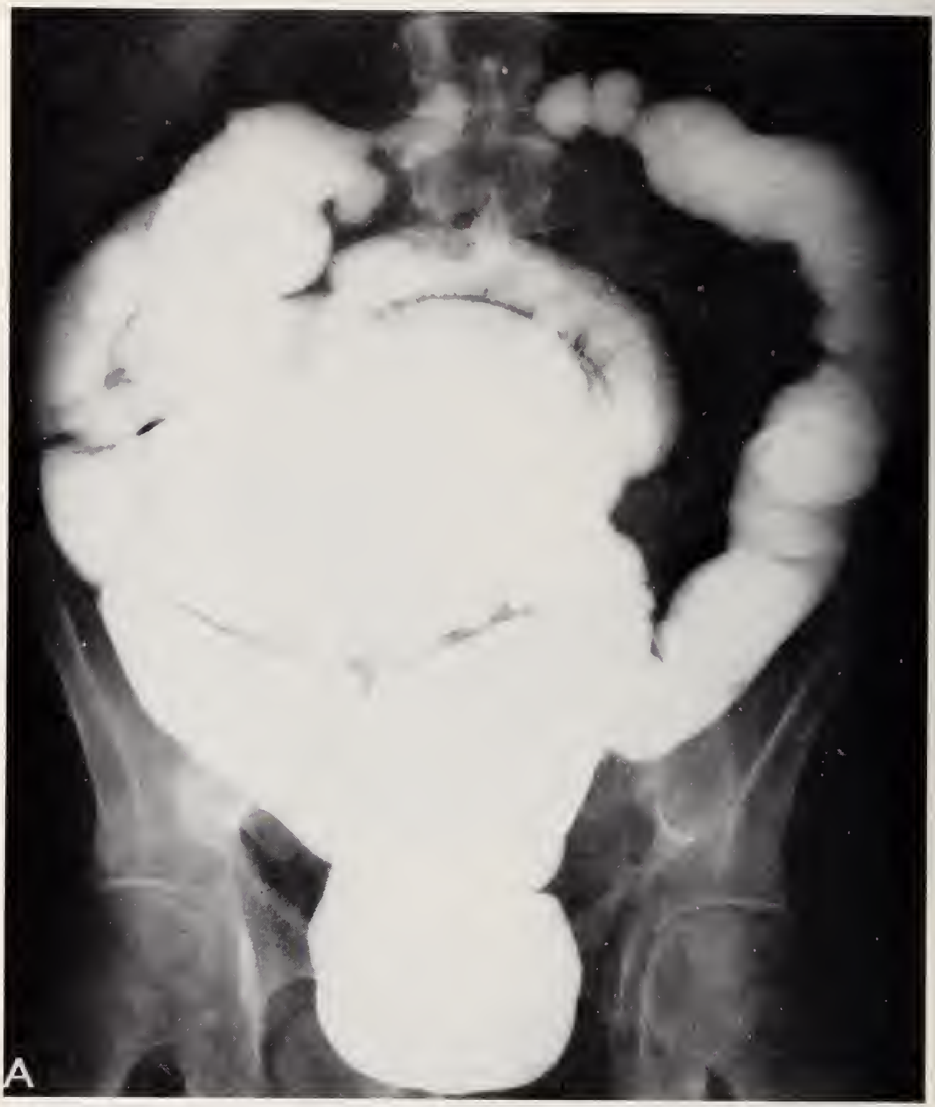
Case 383, Fig. 1B

ulcerations (Fig. 1 A). The pyloric channel was patulous and the duodenal bulb was normal. The upper two-thirds of the stomach were normally distensible. These findings were confirmed to be of a constant nature, (Fig. 1 B) as the narrowed gastric antrum was again demonstrated on pressure spot films. When air filled the antrum, it was seen to be markedly less distensible than the proximal portion of the stomach (Fig. 1 C). Barium enema revealed a normal rectum, sigmoid and distal descending colon (Fig. 2 A). Numerous pseudodiverticula were seen along the transverse colon, which replaced the normal haustrations. Distensibility of the ascending and transverse colon was markedly impaired.

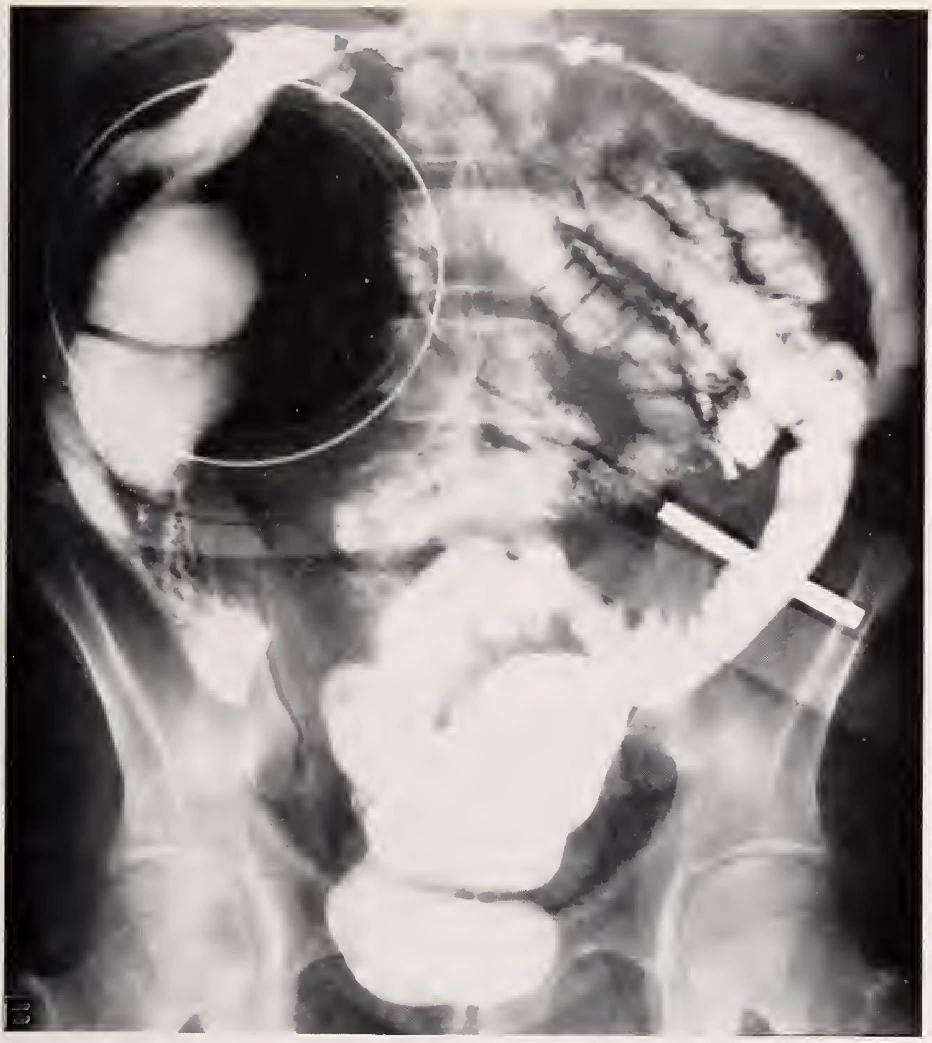


Case 383, Fig. 1C

A large amount of barium refluxed into the distal ileum. When pressure was applied to the right epigastrium, the foreshortened and deformed ascending colon with large pseudodiverticula and cobblestoning of the terminal ileum were clearly visible (Fig. 2 B). In spite of the extensive granulomatous bowel in-



Casa 383, Fig. 2. A, Barium enema in the posterior anterior view reveals a normal rectum, sigmoid and descending colon. The transverse colon is narrowed with pseudo-diverticular formation and effacement of the normal folds. There is marked foreshortening of the right side of the colon. B, Spot film of the right side of the colon reveals narrowing and pseudo-diverticular formation in the right side as well with narrowing and thickening of the folds in the distal ileum.



Case 383, Fig. 2B

volvement, the patient has been able to lead a normal life and has been largely free of gastrointestinal symptoms.

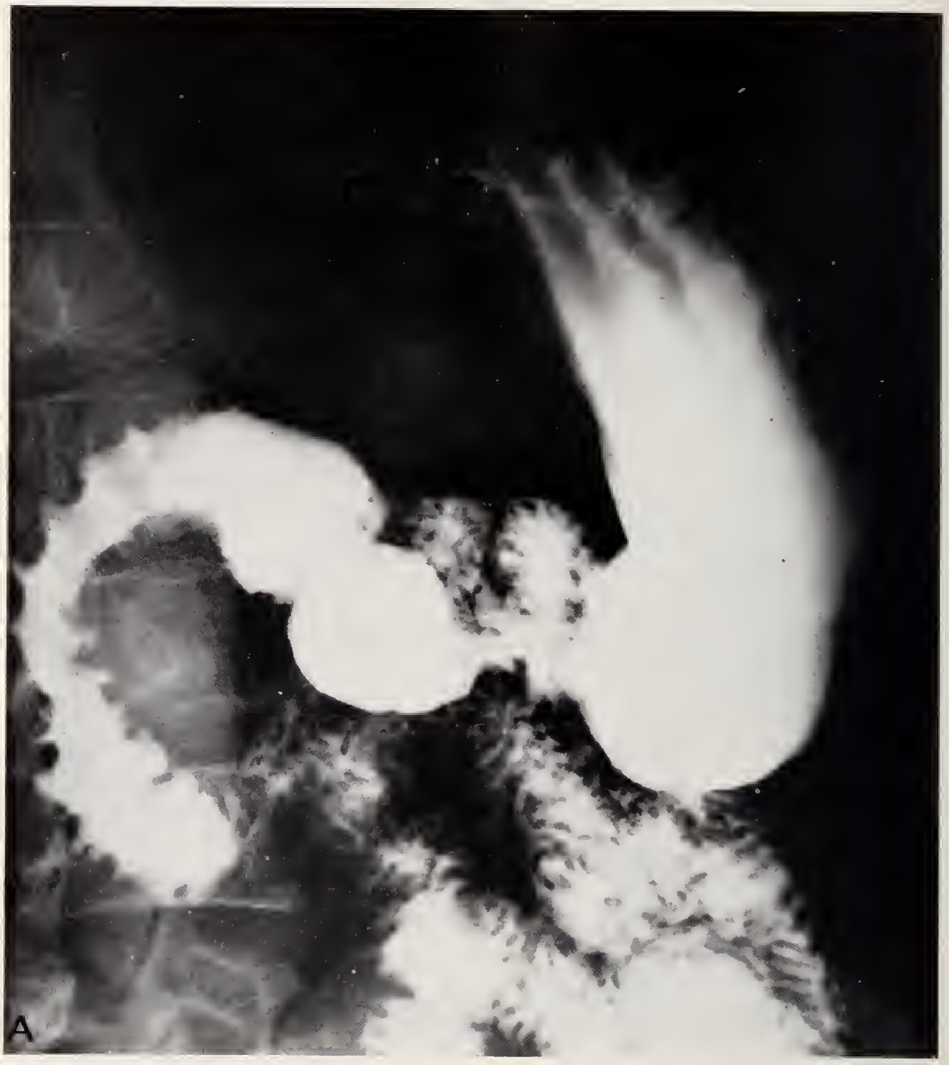
Case Report: CROHN'S DISEASE OF THE STOMACH

Acknowledgment

The editors wish to thank Dr. Lester R. Tuchman and Dr. Charles M. Newman for permission to publish this case.

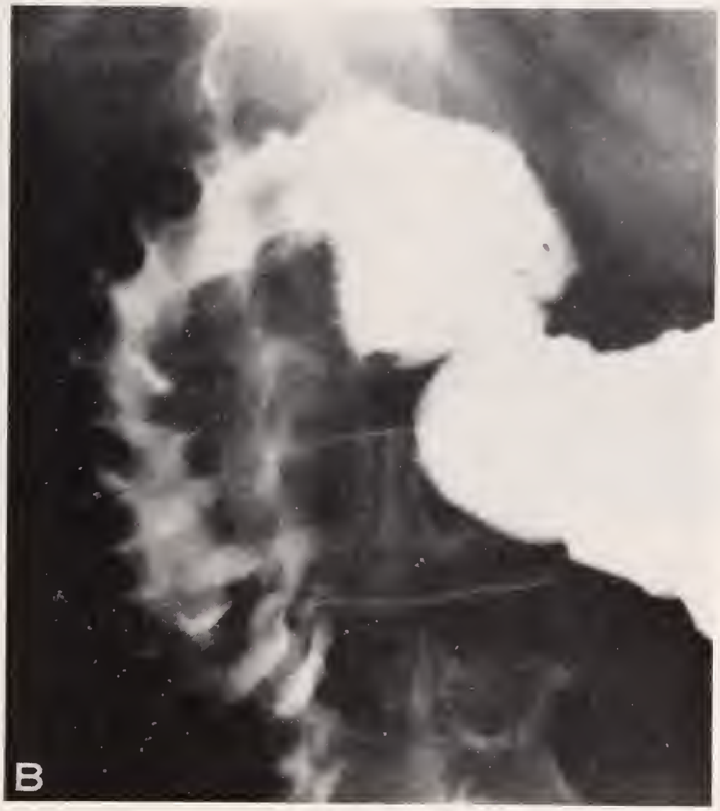
Case No. 384

Because of recurrent bouts of crampy abdominal pains, intermittent diarrhea and weight loss, this 55-year-old woman underwent radiologic examination of

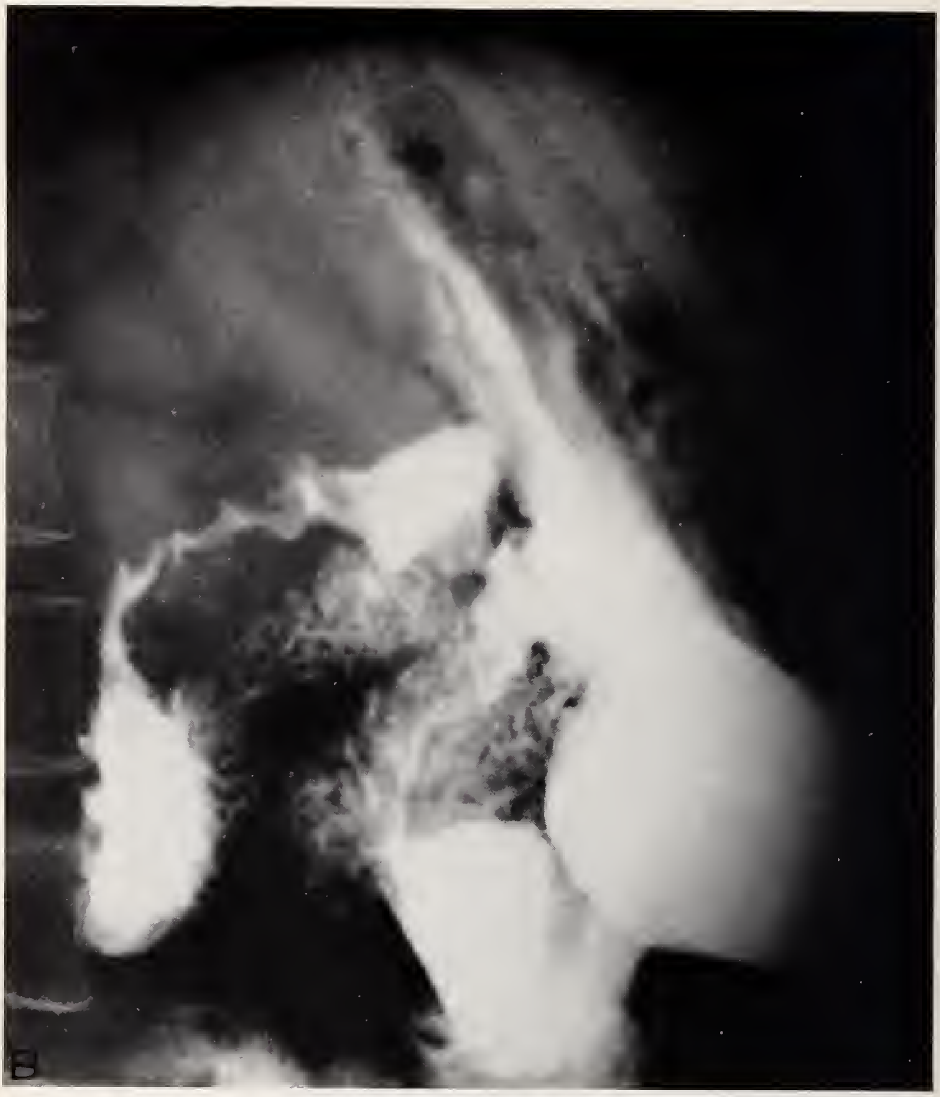


Case 384, Fig. 1. A, Right anterior oblique view of the barium-filled stomach and duodenum reveals no intrinsic abnormalities of the stomach. The folds within the distal portion of the duodenal bulb and in the second portion of the duodenum are markedly thickened and distorted without evidence of ulceration. The third portion of the duodenum and the ligament of Treitz are normal. B, Pressure spot film of the duodenum confirms the presence of the markedly distorted thickened folds and hypersecretion in the second portion of the duodenum, but no ulceration. C, In the right lateral view, there is further evidence of a markedly distorted second portion of the duodenum with narrowing and nodular folds.

the gastrointestinal tract. Gallstones were demonstrated, and a GI series revealed marked thickening of the folds of the duodenum and hypersecretion, which at that time were interpreted as evidence of adjacent pancreatitis. A small bowel series was not performed at that time. The patient was placed on a medical regime for cholelithiasis and pancreatitis, without real improvement in her symptomatology. Physical examination of the abdomen was negative. Laboratory tests showed a moderate elevation of the sedimentation rate and a mild malabsorption with low creatinine, cholesterol and glucose levels. Hemogram and urinalysis were both normal. Repeat gastrointestinal examination revealed the stomach to be normal in contour and distensibility, with preservation of a normal rugal pattern. The duodenal bulb contained markedly thickened and distorted folds especially near the apex (Fig. 1 A). Although the lumen of the descending duodenum remained normal, the mucosal folds there assumed a cobblestoned appearance with an almost nodular character. The constancy of this distorted nodular thickening was demonstrated on a pressure spot film (Fig. 1 B). The distal portion of the sweep and jejunum were normal. In the lateral

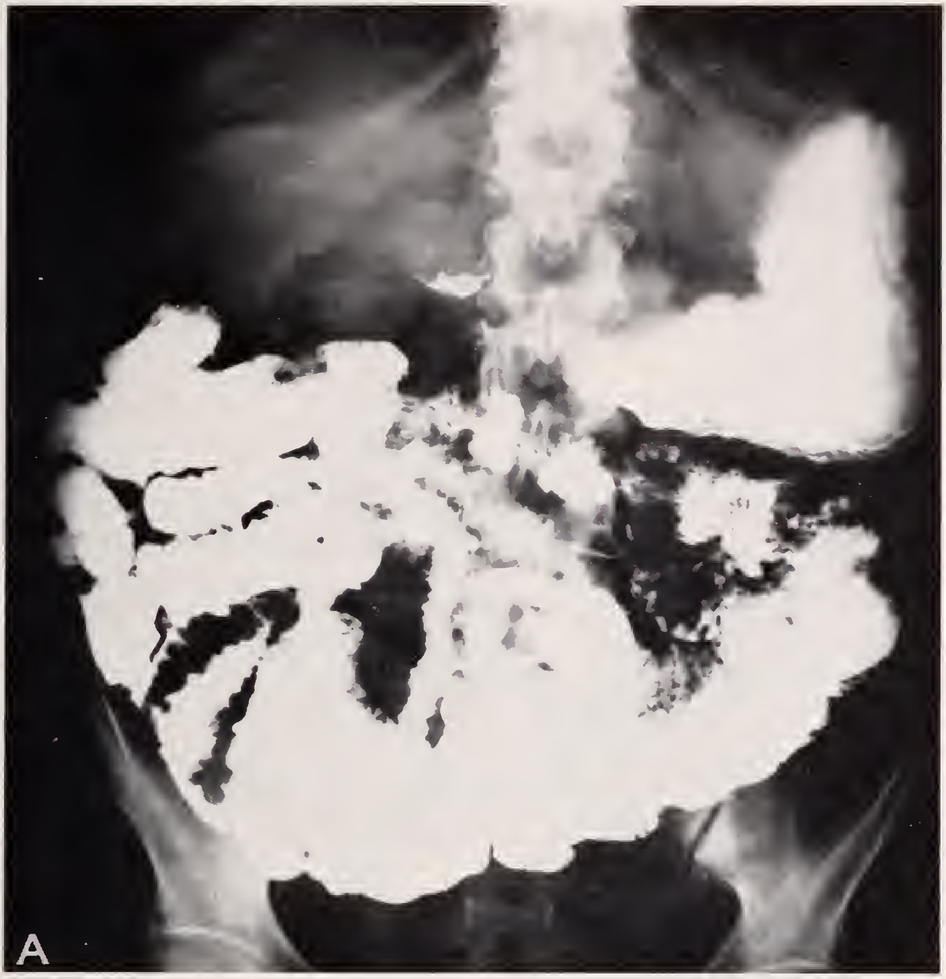


Case 384, Fig. 1B

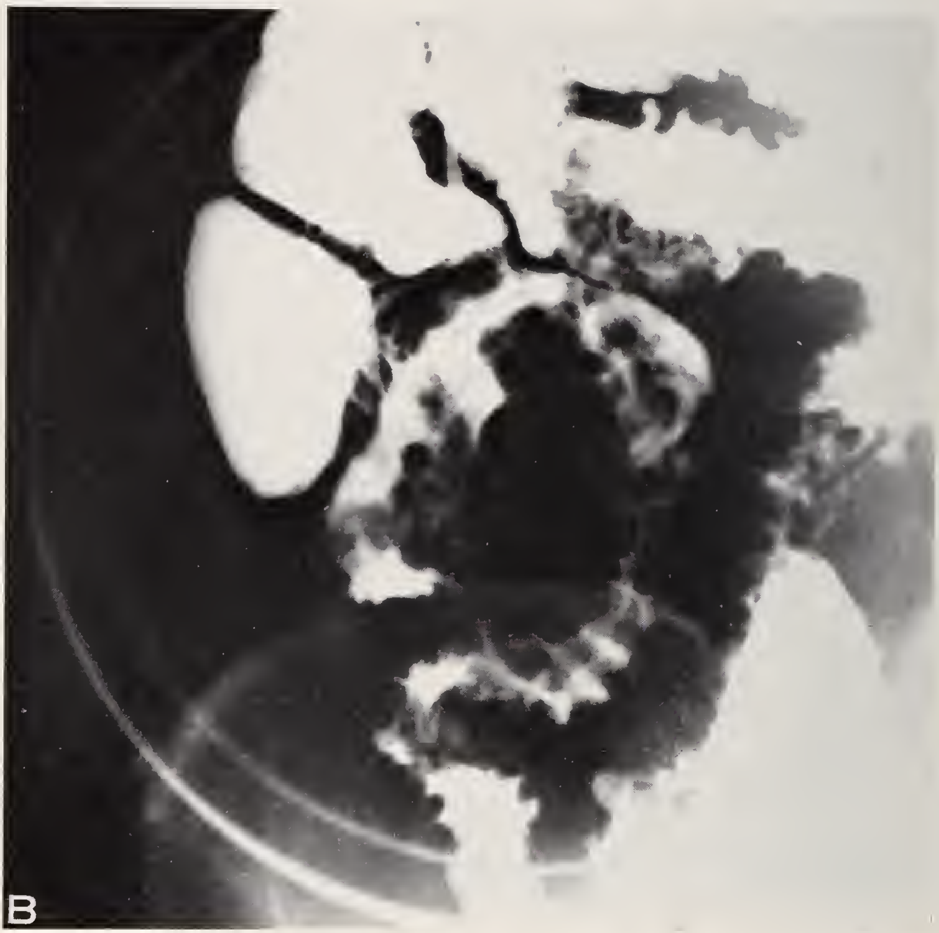


Case 384 Fig. 1C

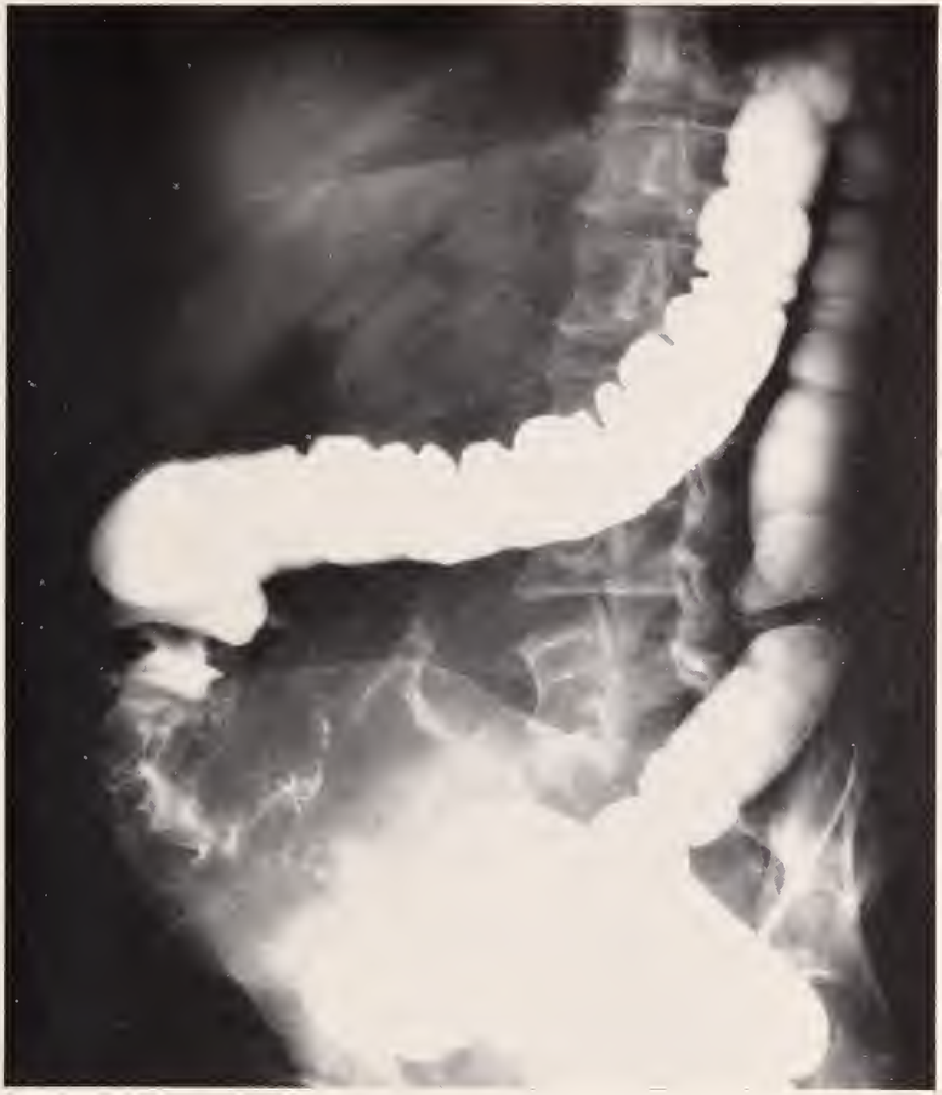
view, the duodenum was noted to be markedly deformed with considerable distortion of the mucosa (Fig. 1 C). The small intestine was the seat of extensive granulomatous disease within the distal two feet of the ileum, with thickened folds, narrowed irregular lumen and separation of the affected loops in the right lower quadrant (Fig. 2 A) Pressure spot film of the area demonstrated the cobblestoned mucosa and numerous fine irregular barium-filled channels (Fig. 2 B), which represented internal fistulae. Barium enema demonstrated involvement of the ascending colon with lack of distensibility, thickened irregular folds



Case 384, Fig. 2. A, The small bowel reveals involvement of the distal third of the ileum with narrowing, distortion of the folds with "cobblestoning" and separation of the loops in the right lower quadrant without ulcerations. B, Pressure spot film of the right lower quadrant confirms the "cobblestoning" of the terminal ileum and distal ileum and separation of the loops. Small barium-filled channels represent internal fistulae.



Case 384, Fig. 2B



Case 384, Fig. 3. Left anterior oblique view of the barium filled colon with reflex into the terminal ileum reveals lack of distensibility of the ascending colon with thickened and distorted folds. Small barium-filled tracts are noted between the medial aspect of the ascending colon and the distal loops of ileum. These undoubtedly represent fistulae.

and fistulae between the medial wall of the ascending colon and loops of small bowel in the right lower quadrant (Fig. 3). The transverse colon was also abnormal in that the haustral markings were asymmetrically flattened inferiorly. The patient's symptoms responded well to medical therapy.

Acknowledgment

The editors wish to thank Dr. Lawrence Schwartz for permission to publish this case.

Discussion of Cases Nos. 383 and 384

Granulomatous involvement of the stomach or duodenum is rare and is almost always accompanied by Crohn's Disease of the small bowel (1). The reported incidence of gastric involvement ranges between 1% and 4% of cases of Crohn's Disease (2, 4).

Symptoms are usually related to the concomitant small bowel involvement, namely diarrhea, cramps and fever. Only when the stomach or duodenum is severely involved and narrowed, does the patient present with nausea, vomiting and dyspepsia (3). Radiologically, the stomach is seen to be involved especially within its distal portion by narrowing and decreased distensibility. The differential diagnosis rests mainly between Boeck's sarcoid, syphilis, and scirrhous carcinoma.[5] When the duodenum is affected, the folds are markedly thickened and there is hypersecretion without ulceration. The differential diagnosis is with peptic ulceration and duodenitis secondary to pancreatitis or cholelithiasis.

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/P.A. — Liver
Hypothyroidism → T4

— wt loss
Gastric Ca

Tx — abrupt —

↓ H ↑ ⇒ ↓ [illegible]
↑ Estr
↑ IgG ↓ IgA
Muscle weakness

Editorial

September 1st will undoubtedly be a day in which the direction of the Medical School will have been profoundly affected by the appointment of three key men: Dr. Richard Gorlin as Murray M. Rosenberg Professor and Chairman of the Department of Medicine; Dr. Arthur H. Aufses, Jr., as Franz W. Sichel Professor of Surgery and Chairman of the Department; and Eoin Aberdeen as Professor of Surgery.

The Chairs of Surgery and Medicine, occupied for the first time in approximately two years, are, of course, pivotal and crucial influences in the Medical School. Dr. Aberdeen brings to the Institution unique experiences in medical education and pediatric cardiac surgery.

The Journal salutes the arrival of these three great educators and physicians.

David A. Dreiling, M.D.

Caplan's Sx
Pneumoconiosis + Rheum. nodules.

Eoin Aberdeen, M.D.

The first of June signaled the arrival of a most distinguished addition to the Department of Surgery's Division of Cardiothoracic Surgery. On that date, Dr. Eoin Aberdeen began his duties in the Division as Professor of Surgery and Director of Pediatric Cardiothoracic Surgery.

Dr. Aberdeen was Australia born and bred and received his medical education at the University of Melbourne Medical School. From student days, Dr. Aberdeen evidenced great interest in pediatric surgery, particularly in relation to problems associated with neonates, trauma and thoracic surgery. His training period spanned fourteen years and included study in world-renowned institutions such as the Royal Children's Hospital in Melbourne, hospitals in England including the Burns Unit of the Birmingham Accident Hospital, the Hospital for Sick Children, Great Ormond Street, London, and in the United States the Stanford University and Presbyterian Medical Center in San Francisco. When queried about his lengthy training, Dr. Aberdeen indicated that it was not entirely that he might be a "slow learner" but, rather, it was more in the British tradition of things.

In 1963, Dr. Aberdeen was appointed Consultant Thoracic Surgeon to the Hospital for Sick Children in London and was given primary responsibility for the development of open heart surgery there. The effectiveness of his leadership is a matter of history. Within a short time The Hospital for Sick Children had become a referral center for the management of complex pediatric cardiac surgical problems for the European continent. Dr. Aberdeen hastens to emphasize the excellent level both of pediatrics and anesthesiology which were such essential parts of that team. Moreover, the outstanding pediatric nursing at the Hospital for Sick Children is regarded as a model not only in Europe but throughout the world. The surgical results attained by this distinguished group became the gold standard of what could be accomplished in correcting severe cardiac defects in infants and children. The leader who orchestrated that program was Eoin Aberdeen.

Dr. Aberdeen comes to The Mount Sinai Medical Center after a period of three years at The Children's Hospital of Philadelphia where he was Director of Cardiac Surgery and Professor of Surgery at the University of Pennsylvania School of Medicine. He brings to Mount Sinai interests much broader than surgery. Together with other faculty members, Dr. Aberdeen hopes to explore new means of exchanging ideas to make available the contents of the literature to the physician and student at the bedside. Dr. Aberdeen suggests that memory is not adequate for resolving many clinical problems; clinical decisions should be formulated by an analysis of up-to-date factual information. This, in turn, requires a categorized system of recall which can be achieved only through means such as computers. Together with Dean Chalmers, Dr. Aberdeen and others are already at work on this problem.

Dr. Aberdeen is a member of many distinguished organizations and societies including The Royal College of Surgeons (England), The Society of Thoracic and Cardiovascular Surgeons of Great Britain and Ireland, The Society of Thoracic Surgeons, The American Association for Thoracic Surgery, and The American Surgical Association.

Dr. Aberdeen brings to Mount Sinai a warm and gentle wit, a kindly and gentlemanly manner, and a respectful concern for all. We welcome him not only for his world-recognized competence but equally important for his humanitarian nature.

Arthur H. Aufses, Jr., M.D.

Dr. Arthur H. Aufses, Jr., Franz W. Sichel Professor of Surgery and Chairman of the Department of The Mount Sinai School of Medicine and Director of the Department of Surgery and Surgeon-in-Chief of The Mount Sinai Hospital, effective September 1, is returning to Mount Sinai after serving for three years as Director of Surgery at Long Island Jewish-Hillside Medical Center and Professor of Surgery at the State University of New York at Stony Brook. He had been Attending Surgeon and Clinical Professor of Surgery at Mount Sinai, rising through the ranks from his initial appointment in 1954 as Assistant Resident Surgeon. A graduate of Columbia University College of Physicians and Surgeons in 1948 at the age of 22, Dr. Aufses trained for four years at Presbyterian Hospital and saw two years of active service in the Army Medical Corps before joining the Mount Sinai staff.

Primarily an abdominal surgeon, Dr. Aufses is best known for his work in surgical management of inflammatory bowel disease, a subject on which he has written extensively. He is a Fellow of the American College of Surgeons, and, since 1958, a Fellow of the New York Academy of Medicine. Dr. Aufses served as secretary of the New York Surgical Society from 1971 to 1973. He is a member of the Allen O. Whipple Surgical Society and the New York Academy of Sciences as well as a member of the scientific advisory board of the National Foundation for Ileitis and Colitis.

Dr. Aufses' association with Mount Sinai is in a family tradition. His father, now Clinical Professor Emeritus of Surgery, has been associated with the institution since 1921. His mother was for many years editor of the publication of the Associated Alumni of The Mount Sinai Hospital. The younger Dr. Aufses first worked at Mount Sinai in 1942 at 16 years of age as an operating room orderly. Mount Sinai proudly welcomes this distinguished surgeon, returning to us now as head of Surgery.

Richard Gorlin, M.D.

Dr. Richard Gorlin, Murray M. Rosenberg Professor and Chairman of the Department of Medicine of Mount Sinai School of Medicine and Director of the Department and Physician-in-Chief of The Mount Sinai Hospital, comes to Mount Sinai from Peter Bent Brigham Hospital in Boston where for sixteen years he was Chief of the Cardiovascular Division, and from a professorship in Medicine at Harvard Medical School. His association with Harvard dates back to 1943 when he entered as an undergraduate. With the exception of two breaks—for one year as Honorary Associate in Medicine at St. Thomas' Hospital in London and two years in the United States Navy—this will be Dr. Gorlin's first appointment away from Boston.

Dr. Gorlin was a member of the National Heart and Lung Institute's Heart Program Task Force in 1967 and, since 1968, has been a member of the Program Planning Committee of the NHLI Myocardial Infarction Research Units, and, since, 1972, of the NHLI Coronary Heart Disease Task Forces. He also served on the Cardiovascular Study Section of the National Institutes of Health from 1965 to 1969 and the Training Grant Committee of the NHLI from 1969 to 1973.

Dr. Gorlin is vice president of the American College of Cardiology and president-elect of the Massachusetts Heart Association. He served as president of the New England Cardiovascular Society in 1967 and 1968. He is a member of the Association of American Physicians, American Society for Clinical Investigation, American Physiological Society, American College of Physicians and the Royal Society of Medicine. He has served on the editorial boards of *American Journal of Cardiology, American Journal of Medical Sciences, Circulation* and *American Journal of Medicine.*

Dr. Gorlin was a member of the Scientific Council of Coeur et Medicine Interne in Paris in 1970 and since 1973 has been a corresponding member of the British Cardiac Society. Early this year he took part in the US-USSR Cooperative Study Program in Heart Disease. In addition to many appearances before professional societies in this country, Dr. Gorlin has recently lectured in the USSR, Great Britain, Argentina, Sweden, Australia and New Zealand.

Heart disease became Dr. Gorlin's principal interest when he was a medical student and tried to create experimental mitral stenosis. This led to an extended study of valvular heart disease as a research fellow, following his internship. This work, involving the application of the principle of hydraulics to human heart valves, is today a standard part of accepted medical science. For more than 20 years since, he has focused his research on various forms of physiological malfunction of the heart. During the last eight years, he has concentrated on clinical, angiographic, and metabolic studies of coronary heart disease, still his major area of investigation.

In his new position Dr. Gorlin will combine new administrative duties with his continuing investigative and teaching interests. We extend our warmest wishes to Dr. Gorlin for a long, productive, and happy career at Mount Sinai.

BARBARA LIPTON, M.D.
1928–1974

In Memoriam

BARBARA LIPTON, M.D.

1928–1974

June 28, 1974

To the residents who are leaving

A teacher's only claim to immortality is her students—and you are mine—the over 200 I have taught in 20 years of medical practice.

I hope that you remember the technical points, the bits of knowledge, the rules of thumb; but most of all I hope I taught you a way of professional life. When you are practicing your specialty the patient must always be uppermost in your thoughts, his welfare the most important thing for you to care about; you must be patient with him and gentle; his anesthetic and surgical procedure may be routine to you, but to him he is experiencing one of the most vital and traumatic days of his life. As an anesthesiologist practicing your profession with skill, you, as a matter of course, will deprive him of his consciousness, respiration, autonomic reflexes. You take him into the great unknown and you are as responsible as any one person can ever be for anyone else, including a parent for a child.

I had always hoped to find a way to bring to the attention of our medical students the exciting ramifications of the specialty of anesthesiology in the area of the acute care—nowhere more acute than in the operating room where immediate decisions and actions are life-saving. Certainly, in areas, the skills learned and employed by anesthesiologists are mandatory. Hopefully, in the future, the utilization of ever more sophisticated monitoring techniques and the advent of assistants to anesthesiologists, be they nurses or other highly trained personnel, will take most of the routine drudgery out of the specialty.

Nowhere is there more opportunity to do clinical research, to see the direct clinical application of the physiologic and pharmacologic principles learned in the laboratory. Nowhere can one observe so closely the pharmacologic actions of drugs given to patients (of necessity for their own well-being) and also use these observations to extend our understanding of these drugs from a research point of view.

I hope I have imbued enough of you with my enthusiasm to go out and proselytize among the medical community, to emphasize the ever-increasing need in our complex medical world for the skills taught in the specialty of anesthesiology and to emphasize the tremendous ramifications of the "Most Acute-Care Specialty" in medicine today.

I wish you all both personal happiness and professional success. Those among you with whom I was privileged to work closely were a comfort and a pride to me during a year of personal struggle. For each of you I have a special affection.

Barbara Lipton, M.D.

Dr. Barbara Lipton

Distinguished Anesthesiologist at Mount Sinai

Dr. Barbara Lipton, Clinical Professor of anesthesiology at Mount Sinai School of Medicine of The City University of New York and Attending Anesthesiologist of The Mount Sinai Hospital died in that hospital on July 20th at the early age of 46.

Dr. Lipton graduated in Arts, Summa cum Laude from Barnard College in 1947 and proceeded to Yale University, New Haven where she received her M.D. degree in 1951. Having interned in Surgery at Yale New Haven Clinical Center she completed her training in Anesthesia at Columbia Presbyterian Medical Center, New York City in 1954.

Following her residency, she was appointed to the staff of Beth Israel Hospital, New York City as Adjunct Anesthesiologist and was promoted to Associate Attending in 1956. During her tenure at the hospital she devoted much of her time to pediatric anesthesia and wrote extensively on the management of small children undergoing major abdominal and plastic surgery.

Leaving Beth Israel Hospital in 1964 she joined the staff of Mount Sinai Hospital as Associate Attending Anesthesiologist and she was promoted to Attending Anesthesiologist in 1972. The inception of the medical school saw her academic qualities fully recognized by her attaining professorial status.

On appointment to Mount Sinai she devoted herself principally to anesthesia for Cardiothoracic Surgery in association with Dr. Robert S. Litwak and the late Howard Gadboys. It was in this field that her sound knowledge of cardiovascular physiology contributed so much to the safe conduct of patients through this complicated surgery.

In addition, in association with Dr. Julius H. Jacobson II, she developed an interest in hyperbaric medicine and until incapacitated by her illness spent many hours in the hyperbaric chamber in productive research into the problems of respiratory failure. When long hours of duty are required to handle such patients, there was no more tireless worker than Barbara Lipton, no one more deserving of seeing her efforts rewarded, no one more upset by failure.

In addition to her busy clinical duties, Barbara Lipton wrote extensively on various aspects of her specialty, calling on her vast experiences to write compellingly and with authority. Her interest ranged through pediatrics, obstetrical and cardiothoracic anesthesia from a clinical standpoint to the more fundamental study of blood gas parameters under various experimental conditions. She was a frequent exhibitor at national meetings and her exhibit, in association with Dr. Litwak on the Management of Anesthesia for Mitral Valve Replacement won first prize at the N.Y.S.S.A. meeting in 1967 and was shown at the World Congress in London in 1968 and the A.S.A. and A.M.A. meetings of 1967.

Her interest in the care of respiratory problems produced an exhibit on this subject, in association with Dr. A. I. Weinreich and Dr. Converse Pierce II exhibited at National Meetings in 1970 and 1971. Her latest exhibit on the use of halothane in thoracic surgery was shown at the A.S.A. meeting in San Francisco in 1972 and was particularly well received at the A.S.A. meeting in 1973.

Dr. Lipton had a fine reputation as a speaker which extended beyond the land of the eastern seaboard and she lectured at various times in Rome, Kyoto, Hong Kong, Paris, Stockholm, Copenhagen, Athens, Oslo, and Rio de Janeiro.

Not content to rest on her laurels as a practitioner, teacher, research worker and internationalist, Dr. Lipton yet found time to engage in society and association work.

She served the New York Academy of Medicine as member and secretary of the admissions committee 1965–1969 and became secretary of the section on Anesthesiology and Resuscitation in 1970 and chairman of the section in 1972.

She served the Medical Society of the County of New York on many important committees such as Public Relations and Nursing Problems and as a delegate to New York State Medical Society for eight years.

The New York State Society of Anesthesiologists benefitted from her dedication and expertise in committee work. She served successively as Secretary and President of District II, as a member and chairman of the Public Relations Committee, became Chairman of the Membership Committee in 1968, and was appointed First Assistant Secretary in 1972.

Among other society interests, she was a member of the A.S.A. and served on subcommittees of that organization and was appointed chairman of the New York Society for Acute Medical Care in 1970. She was a diplomate of the A.B.A. and a Fellow of the A.C.A. which latter body she served as an examiner and became a member of the A.B.A.-A.C.A. liaison committee in 1974.

As a friend and colleague, Barbara was exceptional, always ready with help and advice, exhibiting tremendous patience in teaching juniors and guiding medical students. Her sympathy for her patients was endless and every anesthetic she administered was well considered and skillfully performed.

Barbara loved company, sea voyages and the cultural life of New York City.

Those privileged to work with her will never forget her, nor cease to be amazed by the calm fortitude she displayed during her distressingly painful illness.

Medicine, Mount Sinai and Anesthesia have sustained an irreplaceable loss.

David C. C. Stark, M.D.
for the
Editorial Board

Effects of Ethanol on Liver Morphology and Pancreatic Function in Chronic Alcoholism*

ORLANDO BORDALO, M.D., AMELIA BATISTA, M.D., M. NORONHA, M.D.,
J. LAMY, M.D., AND DAVID A. DREILING, M.D.

The history of alcoholism is as old as mankind itself. In all civilizations alcoholic beverages were widely used. However, in recent times, because of its extent and severity, alcoholism has become a social disease.

Our concept of alcoholism includes those individuals who are alcohol-dependent and in whom chronic alcoholic disease becomes established after prolonged ingestion of amounts of alcohol which surpass the normal human capacity to metabolize it. The alcoholic, then, begins to exhibit characteristic behavior disturbances and to show evidences of organic disease.

In addition to serious psychiatric problems, the toxic effect of alcohol abuse is especially prominent in the GI tract. The liver is considered to be the most affected organ, and liver cirrhosis its most frequent clinical expression. However, previous investigation has demonstrated the high incidence of alcoholism in cases of chronic pancreatitis, and also the frequent association of pancreatic abnormalities with alcoholic liver cirrhosis. Tobon (1), in one necropsy study, reported as many as 47% of chronic alcoholics with pancreatic lesions, most of them chronic. Walter Hess (2), based on data from Dreiling, Howat, Mortiaux, Sarles, Sun and others, affirms that alcohol, as an apparent etiologic factor, appears in about 77% of chronic pancreatitis. Similar conclusions were reached by Comfort and Gambill (3). Banks and Janowitz (4) found fibrosis and pancreatic inflammation more frequent in alcoholic liver cirrhosis than in other types of liver cirrhosis.

We found macroscopic signs of pancreatitis in 16%, pancreatic fibrosis in 14%, "fatty" pancreas in 9%, and brown pigmentation of the pancreas in 10% of the cases, including 3 cases of hemochromatosis, in a series of 103 necropsies of patients who died with alcoholic liver cirrhosis.

Alcohol may or may not cause injury, separately or simultaneously to both organs, depending upon the influence of other factors such as: pattern of alcohol intake, diet, genetic or constitutional predisposition, etc. However, clinically, these two forms of disease are mutually exclusive, i.e., in patients with manifest cirrhosis, there is no clinical evidence of pancreatitis and, conversely, in patients with chronic alcoholic pancreatitis, the liver is spared (4).

The liver is well assessed by biopsy, and morphology is still the best parameter for its study. On the other hand, the most accurate method of evaluating the effect of alcohol intake on the human pancreas has been the study of pancreatic

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From the Department of *Propedêutica Médica* of the *Hospital de Santa Maria*, and *Instituto de Anatomia Patológica* of the Faculty of Medicine of the University of Lisbon.

* This paper was delivered as the Annual Ralph Colp Lectureship at a combined meeting of the Departments of Surgery and Medicine and Division of Gastroenterology, The Mount Sinai Hospital and School of Medicine, New York City, February 7, 1974.

function. Therefore, in our Department of Gastroenterology, we conducted a study to evaluate the effect of the duration of alcohol intake and of the daily consumption on both liver morphology and pancreatic function. The investigation was performed on 213 chronic alcoholic males without complaints of liver or pancreatic disease, undergoing detoxication treatment in a specialized center. Interviews were conducted to estimate their economic status and the socioeconomic implications of their alcoholism (Fig. 1). The type of beverage, (mainly wine, beer and *aguardente*) the daily amount and duration of alcohol intake were carefully evaluated. The daily amount was converted into grams of ethanol (Fig. 2).

We divided our cases into three groups: 1. Those whose daily ingestion of ethanol was less than 160 grams; 2. Those whose daily ingestion varied between 160 and 320 grams; and 3. Those who ingested more than 320 grams of ethanol per day. We also divided the duration of alcoholism into three groups: less than 10 years; between 10 and 20 years; and over 20 years of consumption. The data indicates that more than half of the patients drank up to 320 grams of ethanol per day for a period of more than 10 years (Fig. 3).

In study of the liver, clinical, functional and morphologic data were correlated. Liver biopsy was performed transthoracically, with a Menghini needle, in 103 patients and specimens were classified as follows (Fig. 4). The overall severity

		Number of Cases	%
Absenteeism		126	59
Hospitalizations	Acute intoxication	36	17
	Chronic intoxication	50	23
Marital problems		127	60
Accidents		65	30.5
Arrests		31	14.5

FIG. 1. Social implications of alcoholism (inquiry made on 213 chronic alcoholics).

Type of Beverage	Percentage of Ethanol	Amount Corresponding to 160 grams of Ethanol
Wine	10–12%	1.780–1.480 L
Beer	5%	3.560 L
"Aguardente de Bagaco"*	40%	0.440 L
Brandy	40%	0.440 L
Whisky	40%	0.440 L

* Portuguese colorless brandy.

FIG. 2. Amounts of common alcoholic beverages containing 160 grams of ethanol.

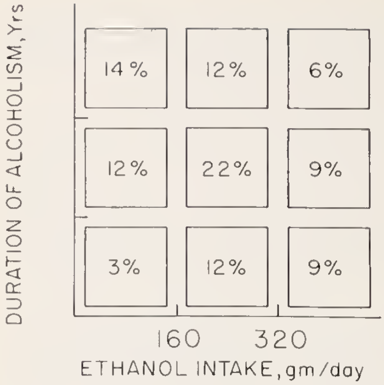


FIG. 3. Relationship between amount of alcohol consumed daily and duration of intake (213 chronic alcoholics).

Normal			
Steatosis	Mild Moderate Severe	<30 30–60 >60	Hepatocytes per field
Fibrosis Portal Parenchymal	Mild Moderate Severe		
Inflammation Portal Parenchymal	Mild Moderate Severe		
Alcoholic Hepatitis			
Cirrhosis			

FIG. 4. Liver morphology parameters.

of morphologic changes was arranged in three categories: 1. normal, 2. mild alterations, and 3. moderate and severe alterations.

A large number of cases with morphologic abnormalities were found (Fig. 5). Steatosis was seen in 76%, inflammation in 73% and fibrosis in 67% of the cases. These three pathologic findings were combined in 52%; signs of alcoholic hepatitis were evident in 12 cases (12%); and cirrhosis in 2 cases. In general, the severity of steatosis declined with the lengthening of hospitalization and, consequently, with the implicit duration of abstinence. Central fat granulomas were found in 24% of the cases, fat cysts were rare, and fat necrosis was not present. Sections showing normal liver, inflammation or fibrosis were most numerous in the liver biopsies obtained late in the course of hospitalization. Small inclusions

Number of Cases (103)
- Normal (13)
- Abnormal (90)

Steatosis	8
Steatosis + inflammation	12
Steatosis + fibrosis	5
Steatosis + fibrosis + inflammation	54*
Fibrosis + inflammation	10**
Fibrosis	1

* 11 Cases with alcoholic hepatitis and 2 cases with cirrhosis are included.
** 1 Case with alcoholic hepatitis is included.

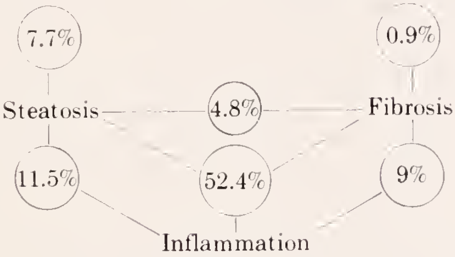


FIG. 5. Morphologic abnormalities in liver biopsy specimens from 103 chronic alcoholics.

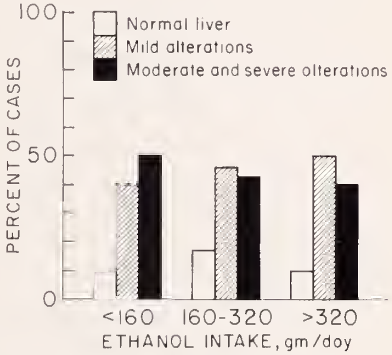
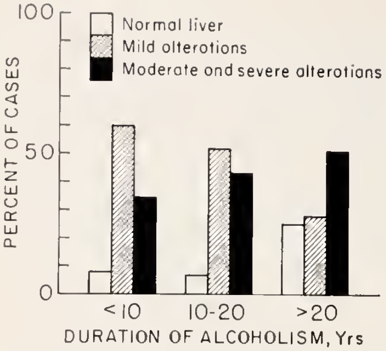


FIG. 6. Relationship between liver morphology and daily intake of ethanol.

of hemosiderin were observed in 6% of the cases. In 37 specimens studied by electron microscopy, the most important alterations pertained to mitochondria and, in general, the findings were similar to those described in the literature.

Pathologic alterations were not related to the amount of alcohol taken daily. We often found severe alterations in group 1 and normal livers in group 3 (Fig. 6). An association between the frequency and severity of pathology and the duration of alcoholism was suggested. A progressive increase in frequency of moderate and severe alterations and, simultaneously, a decrease in frequency of mild alterations was observed (Fig. 7). However, severe fibrosis and cirrhosis were encountered only when daily intake exceeded 160 grams of ethanol for more than 10 years.

In a study of 50 patients with confirmed alcoholic cirrhosis, similar relation-



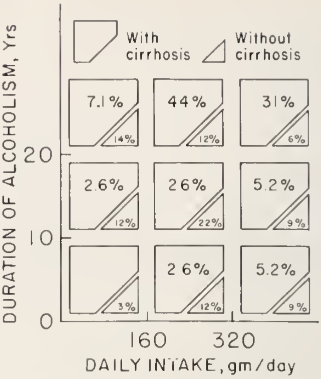


Fig. 7 (*left*). Relationship between liver morphology and duration of alcoholism.
Fig. 8 (*right*). Correlation between duration of alcoholism and daily intake in 50 alcoholic cirrhosis of the liver.

Note: Percentages corresponding to chronic alcoholics without cirrhosis are in the small triangles in the lower right corners.

ships were found. Cirrhosis was not observed in patients who consumed less than 160 grams of ethanol daily for less than 10 years. The higher incidence of cirrhotics was seen in patients drinking more than 160 grams of ethanol daily for more than 20 years (Fig. 8). Liver function revealed that B.S.P. retention, prothrombine time and serum transaminases activity were, in decreasing frequency, the tests which most frequently showed abnormal results (Fig. 9). In the pathologic material, only B.S.P. retention and elevation of serum transaminases activity correlated with the extent of hepatic lesions (Fig. 10).

In relation to the liver, the data can be summarized as follows: 1) Hepatic toxicity is lower than the 160 grams daily consumption considered by some investigators (5, 6) as the threshold for hepatic toxicity. A possible explanation for this discrepancy might be due to differences in nutritional status or genetic background; 2) Liver damage can occur in the absence of biochemical alterations; 3) Morphologic abnormalities correlated best with duration of alcoholism; 4) Severe fibrosis and cirrhosis seem to be both time- and dose-dependent.

Following this investigation, we evaluated pancreatic function in 43 subjects randomly selected from the same group of 213 chronic alcoholics. The protocol of study was a sequential test using 1 and 5 U/Kg of "Boots" secretin and a duodenal collection of 80 min for each stimulation (7, 8). The analyzed parameters were:

1) Volumes—ml/Kg
2) Maximal bicarbonate concentration—mEq/l
3) Bicarbonate secretion—mEq/80 min.

In general, the majority of cases revealed normal pancreatic function (64%). With regard to the abnormal responses, a pattern of qualitative insufficiency was observed in 6% and, in 30% of cases, high volumes were obtained in duodenal collections. As a result, three patterns of pancreatic response, in the presence of alcoholic injury, were encountered:

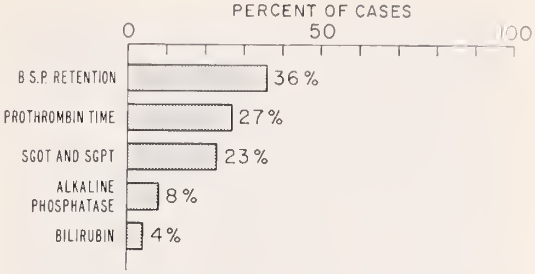


FIG. 9. Percentages of abnormal results of laboratory tests in chronic alcoholics.

Degree of Morphologic Changes	Number of Cases	Abnormal Results of Tests		
		B.S.P. retention	Prothromb. time	Transaminases
Normal liver	8	25%	13%	13%
Mild alterations	35	37%	26%	20%
Moderate and severe alterations	31	45%	16%	32%

FIG. 10. Relation of abnormal results of laboratory tests to morphologic changes in the liver.

1) *Normal function,* i.e., when volumes and bicarbonates were normal.
2) *Hypersecretion,* i.e., when volumes were above the statistically calculated upper limit of normal. Maximal bicarbonate secretions were normal or slightly decreased.
3) *Insufficiency,* qualitative, i.e., when low bicarbonate concentrations and normal volumes were obtained or total, i.e., when volumes and bicarbonate concentrations were decreased.

In our series it was not possible to establish a correlation between abnormal function, daily amount and/or length of time of consumption of ethanol. However, abnormal function did correlate best with daily amount, since pancreatic insufficiency was only observed in subjects drinking more than 160 gm of ethanol per day (Fig. 11). A high percentage of abnormal pancreatic function was observed in a series of 30 cases of confirmed alcoholic liver cirrhosis, in which 75% of the subjects drank more than 160 grams of ethanol daily for more than 20 years. Comparing these two groups of alcoholics, with and without liver cirrhosis, a lower incidence of normal function and a higher incidence of pancreatic insufficiency in the group of cirrhotics, is evident (Fig. 12). Hypersecretion was the most frequent abnormality found. The data thus suggests that:

1) The establishment of pancreatic insufficiency is time- and dose-dependent.
2) Pancreatic hypersecretion is the most frequent functional abnormality in chronic alcoholism.

Although hypersecretion was reported 25 years ago in patients with liver cirrhosis, little attention has been paid to the hypersecretory states of the pancreas. Physiologists always directed their interest towards deficiency patterns. However, today's purified and well-standardized secretin preparations make possible

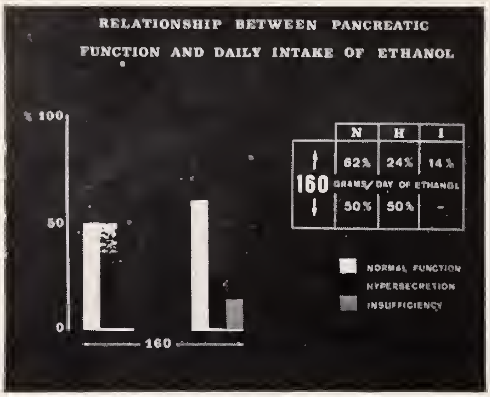


FIG. 11.

	Without Liver Cirrhosis	With Liver Cirrhosis
Normal	64%	6%
Hypersecretion	30%	83%
Insufficiency	6%	11%

FIG. 12. Pancreatic function in chronic alcoholics.

the establishment of reasonable criteria for the upper limits of secretion with pancreatic tests.

The high volumes collected in the duodenum of alcoholics without cirrhosis directed our attention to the comparison of the pancreatic function in the different groups of hypersecretors (Fig. 13 and Fig. 13A):

1) With both stimulation dosages, the cirrhotics displayed marked flow increases, and the alcoholics without cirrhosis secreted at rates greater than normal subjects, but lower than cirrhotics.

2) Cirrhotics and alcoholics without cirrhosis, displayed both maximal bicarbonate concentrations lower than those observed in normal subjects, but the depression was greater in cirrhotics than in non-cirrhotics.

3) Bicarbonate secretions were lower than the normal means in both groups, but were markedly increased after augmented stimulation, suggesting that the depression of bicarbonate concentration was due to the dilution.

4) In alcoholic pancreatitis all parameters were decreased.

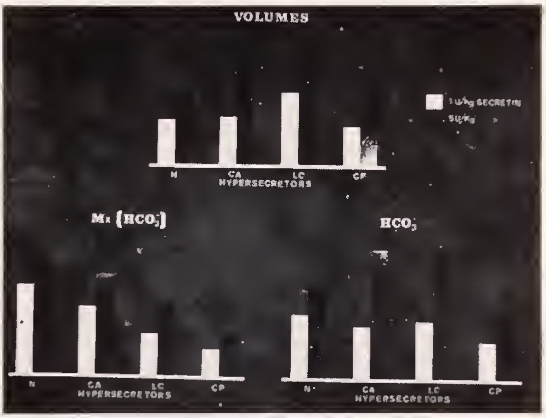
These data indicate that the high volumes collected in the duodenum of patients with chronic alcoholism without cirrhosis were, indeed, due to pancreatic hypersecretion. These volumes became higher in cirrhotic patients, due to the known participation of the liver, i.e., increased bile flow, either by canalicular reduplication or by augmented sensitivity of ductal cells to secretin (9) or both.

Pancreatic hypersecretion, thus, could be the initial post-secretin pattern of minimal pancreatic inflammatory pathology, resulting from the reaction of the pancreas to injury, namely, ductular reduplication, hypertrophy and/or hyper-

		Standard			Augmented		
		Vol ml/kg	$Mx(HCO_3^-)$ mEq/l	HCO_3^- mEq/80	Vol ml/kg	$Mx(HCO_3^-)$ mEq/l	HCO_3^- mEq/80
Normals	30	3.1	113	21.6	4.9	129	31.5
Chronic alcoholics hypersecretors	40						
Without cirrhosis 14		3.4	99	17.5	7.4	120	45.2
With cirrhosis 26		5.1	78	19.5	8.9	99	44.5
Alcoholic chronic pancreatitis	40	2.8	69	12.7	4.2	80	22.6

FIG. 13. Postsecretin data of chronic alcoholic hypersecretors (means).

FIG. 13*A*



plasia, a response which was histologically confirmed by Dreiling, as well as ourselves (10, 11). As the inflammation progresses, the more advanced pathologies namely, fibrosis, atrophy and calcification, lead to a marked fall in all parameters, resulting in a secretory pattern characteristic of chronic pancreatitis.

Sarles reported canalicular reduplication due to neogenesis, "protein plugs" and increased "proteinaceous" secretion in alcoholic rats (12). This investigator also observed a reduction in the amount of acinar parenchyma, thus, in agreement with Löffler (13) who considered the depression of amylase secretion the most significative parameter as the initial expression of pancreatic abnormalities in chronic alcoholism. However, in our series of alcoholics, normal amylase secretion was found after pancreatic stimulation with secretin and pancreozymin.

Discussion

As far as the liver is concerned, morphology and metabolism of alcoholic disease have been thoroughly investigated with great contributions made to the understanding of the physiopathology of this condition. That alcoholic steatosis per se is the precursor of cirrhosis is rather doubtful. This finding is common in heavy drinkers who never exhibit morphologic signs of cirrhosis. It is likely, therefore, that most chronic alcoholics never go beyond the stage of fatty liver, or if the disease progresses, fibrosis develops, which can also be reversible (14). Our data is in agreement with the implications of serial biopsies in man, namely, that cirrhosis is the result of earlier episodes of alcoholic hepatitis (15, 16).

The similarities between liver and pancreas in the presence of alcoholic injury, based on experimental data in rats, recently reported by Edlund (17) and Sarles (12), lead to the hypothesis that, in man, fatty degeneration of pancreatic cells, associated or not with fibrosis, could be the first morphologic response of the pancreas to chronic alcoholic ingestion, a pathologic state still without detectable functional abnormality. Although, the existence of alcohol dehydrogenase was denied by Holt et al (18) and a microsomal ethanol oxidizing system (MEOS) has not yet been reported in the pancreas, the increased number of peroxisomes described by Sarles (19), in rats fed with ethanol, could indicate aberration of an alternative pancreatic oxidizing mechanism. Though we postulate that fatty accumulation in the pancreas may be the result of a direct alcohol injury, we do not deny it might also be caused by the hyperlipemia reported in patients with fatty liver. However, when ductular reduplication, hypertrophy and/or hyperplasia appear, functional abnormalities appear as evidenced by the hypersecretion pattern.

When alcoholics stop drinking, all these morphologic alterations may regress. On the other hand, if they continue to drink, repeated episodes of acute subclinical pancreatitis, could explain the progression to chronic pancreatitis, similar to the pathogenetic sequence in alcoholic liver cirrhosis. Endoscopic pancreatic biopsies, if possible, should provide material for the better understanding of physiopathology of alcoholic pancreatic disease.

Summary and Conclusions

1. Morphologic abnormalities of the liver correlated best with the duration of alcoholism. However, severe fibrosis and cirrhosis seem to be both time- and dose-dependent.

2. Abnormalities of pancreatic function seem to correlate best with the daily amount of ethanol consumed. However, pancreatic insufficiency is also both time- and dose-dependent.

3. Hypersecretion is the most frequent pancreatic functional abnormality in chronic alcoholism, probably due to ductular reduplication, hyperplasia and/or hypertrophy.

4. Possible mechanism for direct toxic effect of alcohol on the pancreatic parenchyma were discussed in an attempt to elucidate pathogenetic mechanism of alcoholism in pancreatic inflammation.

Acknowledgment

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Acid Secretion in Duodenal Ulcer Disease before and after Surgery*

J. H. BARON, D.M.

Our approach to the etiology of duodenal ulcer is still based on the 1910 formula "Acid versus Mucosal Resistance" (1). There is little to suggest that the duodenal mucosa is primarily abnormal in duodenal ulcer and this review will therefore seek to elicit the abnormalities of secretion which may be responsible for the duodenum being abnormally exposed to acid, and thus susceptible to inflammation and ulceration. Additional references can be found in other recent reviews (2–4).

Duodenal Acidity

A duodenal ulcer could develop if the duodenum was exposed to too great a volume of acid output of gastric secretion, too high a mean acidity, or too many periods of high acidity; and these abnormalities may be important insofar as they occur either throughout the twenty-four hours, after meals or during basal secretion. It will be seen from Table I that the available data suggests that on average the contents of the first part of the duodenum (but not the distal parts) are more acid for longer periods than normal under fasting conditions, after meals, overnight, and throughout the twenty-four hours, but the range in duodenal ulcer overlaps the normal range. In many studies the intraduodenal acidity was correlated with the gastric acidity. Both the frequency of fluctuations of nocturnal acidity (5) and the intraduodenal acidity (6) were correlated with the basal and maximum acid outputs of the stomach, so that this increased duodenal acidity may be due to excess acid leaving the stomach quite apart from any inadequate neutralization in the duodenum. These mechanisms will be discussed in turn.

Maximum Gastric Secretion

In patients with duodenal ulcer, measurements of peak acid output in response to maximum doses of histamine, histalog or pentagastrin are about twice the normal, but the overlap is such that about a third of duodenal ulcer have a higher peak acid output at the upper limit of normal, and a third of the control subjects secrete less than the lowest value of peak acid output of a duodenal ulcer (for data see Table XI of Baron) (7). There is thus a threshold of peak acid output of about 15 mmol/h below which patients with duodenal ulcer are seldom found (8).

The hypersecretory situation is an expression of the morphologic situation

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* Special Surgical Lectureship & History of Medicine Seminar delivered at The Mount Sinai Hospital, May 14, 1974.

TABLE I

Intraduodenal Acidity in Control Subjects and Duodenal Ulcer

Time	Method	Part of Duodenum	Timing	pH	Controls		Duodenal Ulcer		P	Source
					N	Result	N	Result		
Fasting	Aspirates	2nd	2-6 h	mean		5.4		3.5		98
				<2	7	3%		43%		
				<4		18%	13	70%		
	Aspirates	2nd	0-30 min	mean	22	5.6	23	4.0		99-102
				<3.5	7	26%		44%		
	Electrode	Descending	A.M.	mean	14	3.9	10	3.6	NS	103
				<4		8.8		6%	NS	
	Electrode	Bulb descending	A.M.	mean	59	4.5	16	2.9	P < 0.01	104
				mean		6.7		6.7	NS	
	Telemetering pH capsule	Bulb descending		mean	24	2.7	22	2.8	NS	105
				mean		3.2		3.4	NS	
	Electrode	Descending		mean	9	6.3	8	6.3	NS	106
				<3		6%		3%	NS	
				>5		87%		88%	NS	
Overnight	Aspirates	1st	6 h	2	15	56%	15	74%		16
	Electrode	Bulb	6 h	% fluctuations	6	32%	9	51%	NS	5
After meal	Aspirates		0-2 h		40	usually alkaline	19	8 occasionally acid		107-108
	Aspirates	Bulb	1 h	<3.5	13	0	6	100%		109
	Aspirates	2nd		mean	22	4.94	23	3.87		
				<3.5		32%		54%		101, 102, 110
	Aspirates	1st	day	2	15	60%	15	90%		16
	Electrode	Apex bulb	60-105 min	mean	10	5.6	9	4.3		
				<2.5		20%		100%		111
		Upper descending		mean	6	5.6	5	5.4		
				<2.5		17%	20	20%		
	Electrode	Proximal descending	0-40 min	mean	8	5.3	9	5.4	NS	106
				<3		4%		2%	NS	
				>5		67%		65%	NS	
			40-100 min	mean		5.2		5.4	NS	
				<3		9%		2%	NS	
				>5		64%		65%	NS	
	Aspirates	Proximal descending	60-90 min	mean	25	5.9	15	5.2	<0.005	6

whereby one half of ulcer-free subjects have parietal cell counts of less than 1×10^9, the lowest number in the 15 stomachs of patients found to have duodenal ulcer at postmortem (9). Unfortunately, there have been no other direct counts of parietal cells at postmortem. Parietal cell counts from total gastrectomy specimens are needed, but this operation is rarely performed for duodenal ulcer except in the Zollinger-Ellison syndrome which is itself atypical. In the one study of four patients with ZE there was no correlation between peak acid or peptic outputs and parietal or chief cell counts (10).

The number of parietal cells is a limiting factor on the utmost capacity of gastric secretion, but in everyday life secretion is basal or postprandial. One unsolved problem of the hypersecretory situation in duodenal ulcer is to what

TABLE II

Diagnostic Discrimination of Basal Secretion with the Proportion of Normal Subjects with Measurements below the Least Value of a Patient with Duodenal Ulcer (L.V.D.U.) and the Proportion of Duodenal Ulcer Patients with Measurements above Upper Limit of Normal (U.L.N.)

Test	Unit	Normal				Duodenal Ulcer				Ref.
		N	Mean	% <LVDU		N	Mean	% >ULN		
Morning										
	mmol/10 min	70		31	0.15	52		19	2.0	112
	♂ mmol/h	137	1.4	0	0	238	2.9	21	5	113
	♂ mmol/h	20	1.3	5	0.1	50	3.6	29	4.5	8, 114
	♂ mmol/h	6.5	2.4	23	0.1	787	5.3	28	?6.6	115
	♀ mmol/h	20	1.1	0	0	20	2.2	20	4.6	8, 114
	♀ mmol/h	634	1.3	31	0.1	245	2.9	25	?4.1	115
12 hr night (after 24 h fast)	♂ mmol	21	20.3	41	12.2	31	61.4	48	58.6	116
	♂ mmol	12	17.6	83	36.5	16	69.7	44	55.1	11

extent any excess secretion before and after meals is due merely to increased parietal cell mass.

Basal Secretion

For most of the day the stomach is secreting during and after meals, but for about a third of the 24 hours an individual is sleeping or awaiting the first meal. Basal secretion is usually collected for one hour in the morning even though the untrained subject and his stomach are obviously not in a basal state. 'Basal' secretion can only represent the secretion of that proportion of the parietal cell mass which is being excited under the conditions of the test and is therefore a measurement of poor repeatability. Measurements of 12-hour overnight secretion are difficult and unreliable, and it will be seen from Table II that they provide little diagnostic advantage over the one-hour morning measurement. About one quarter to one half of duodenal ulcers secrete more than the upper limit of normal, and a variable proportion of control subjects secrete less than duodenal ulcers so that again the ranges overlap. Measurement of 12-hour overnight secretion after a 24-hour fast may be more discriminating (11), and it is possible that the whole 24-hour basal output, or part of its circadian rhythm (12) may prove more discriminating than other measurements of basal secretion.

Food (Table III)

All reported studies with the various test meals used in the last 100 years have shown that higher gastric acidities are commoner than normal in duodenal ulcer, but that the ranges overlap almost completely, and this pattern is also seen in 24-hour pH profiles (13–16). The amount of acid secreted by the stomach in response to a solid meal has been estimated by two different methods. Rune (17–19) measured the base excess in arterial blood, and calculated that

TABLE III

Diagnostic Discrimination of Meal-Stimulated Secretion

Meal	Unit	Normal				Duodenal Ulcer				Ref.
		N	Mean	Proportion <LVDU		N	Mean	Proportion >ULN		
Caffeine	mmol/h	526	34	13%	8	362	67	1%	110	117
Broth	mmol/h	5	6.1	100%	10.5	Inactive 6	12.2	100%	11.0	
				100%	12.1	Active 10	24.8	100%	11.0	24
Steak & toast	mmol/h	6	30	5/6	39	7	64	6/7	41	20

acid secretion in the first hour after a meal was comparable with the maximal histamine response. Fordtran and Walsh (20) used intragastric titration with sodium bicarbonate solution. Peak acid output in the four hours after a meal in the duodenal ulcer was twice normal with little overlap between the two groups. After a meal those with duodenal ulcer had higher peak acid outputs than after maximal histamine stimulation, whereas most of the control subjects secreted less after food than after histamine. Thus this ratio PAO Meal:PAO Histamine (which ratio was significantly correlated with the serum gastrin one hour after a meal) was significantly greater in duodenal ulcer's than in control subjects. Moreover duodenal ulcer patients emptied their meal-buffer twice as fast as normal, again with little overlap, so that the well-known high intragastric (and thus intraduodenal) acidity after a meal may be due to a high parietal cell mass, increased parietal cell responsiveness and increased buffer emptying.

The "Drive" on the Parietal Cells

Basal secretion is correlated with maximal secretion, so that basal hypersecretion in duodenal ulcer can broadly be explained merely on the basis of an increased parietal cell mass. However, there may be an increased "drive" (vagal and/or hormonal) on the parietal cells in duodenal ulcer, since in some series the ratio of basal to stimulated acid output was greater than normal in duodenal ulcer (see Table 2.2 of Baron (2)): in the overlap range of 15–35 mmol/h peak acid output, this ratio was 4.5% in control subjects and (significantly higher) 9.8%, in duodenal ulcer (21).

Not only may basal gastric secretion form a higher proportion of peak output than normal in duodenal ulcer, but activity of the duodenal ulcer may (22) or may not (23) be associated with a higher basal output, whereas peak output is similar in active and inactive duodenal ulcer (24, 25). However, patients with duodenal ulcer who do not have active symptoms produce less than 15 mmol/h in response to a broth meal. The normal mean ratio of acid in response to broth: histamine is 35%, and similar in patients with inactive duodenal ulcer

(32%), but more than twice as high (78%) in patients with active duodenal ulcer (24). This increased antral activity in response to the broth meal may be due to the chemical constitution of the meal, or to distention of the antrum, since distention of a balloon in the antrum may evoke considerable acid secretion in patients with active duodenal ulcer (24, 26). It is possible that antral activity is influenced by vagal cholinergic tone, which may also influence duodenal ulcer activity by another mechanism, since pentagastrin evokes a higher pepsin output in active than in inactive duodenal ulcer (25).

One can alternatively try to assess the proportion of parietal cells which are vagally excitable and Hunt (27) calculated the ratio of acid output in response to insulin and to a maximum stimulus, but could not find in the data of Ihre (28) a significant difference between duodenal ulcer and normal. However, in another study (29) this ratio was significantly higher in duodenal ulcer than in G.U.

A further suggested method of assessing vagal drive is the ratio of pepsin to acid in the gastric juice, as pepsin secretion is said to be more readily stimulated by the vagus than is acid. A proportion of the patients with duodenal ulcer have been classified as "vagal" because of the relative excess of pepsin and those with the higher basal acid outputs as "antral" because of lower pepsin outputs (30, 31).

Parietal Cell Sensitivity

Apart from an increased secretory capacity and a possible increased drive on the parietal cells, there is a third suggested mechanism, namely an increased sensitivity of the parietal cells to stimuli. In dose-response studies of intravenous infusion of graded doses of pentagastrin, the mean dose for half maximal response was 92 ng/Kg in duodenal ulcer and 247 in control subjects, who required between two and three times more pentagastrin than duodenal ulcer patients for equal response expressed as a percentage of highest response (32). This increased sensitivity could be due to increased vagal tone (which is not measurable), to decreased concentrations of inhibitory hormones (which are not readily measurable) or to decreased parietal disposal of pentagastrin (again not measured) (32).

Gastrin

It is possible that patients with duodenal ulcer may secrete abnormal amounts and types of gastrins, but the available data is conflicting and difficult to interpret (33). It is not yet possible to quantify the output of gastrins secreted by the G-cells, and we can only measure the concentration of gastrins in the antral mucosa of the blood. There are many forms of gastrin—minigastrin, little gastrin, big gastrin, big-big gastrin and component I—and each may (II) or may not (I) have the tyrosine sulphated. Most studies have been done by radioimmunoassay using only a single antibody.

There are no consistent differences from normal in the concentration of antral gastrin in duodenal, nor any consensus of opinion as to whether the fasting serum gastrin level in duodenal ulcer's is normal or higher or lower than normal. Patients with duodenal ulcer consistently attained significantly higher serum gastrin levels than control subjects in response to meat meals and to insulin hypoglycemia, even when the peak acid outputs (and presumably the parietal cell masses) of the two groups were the same. Thus patients with duodenal ulcer may have a greater than normal "functional G-cell mass." This could be due to a normal number of G-cells, each releasing more gastrin than normal, or to an increased number of G-cells (in the antrum and or duodenum), each producing normal amounts of gastrin. Counts are needed of the actual number of G-cells in patients with duodenal ulcer. We also need to know whether the forms in which gastrin is secreted in duodenal ulcer are different from normal (34). Duodenal ulcer may well be a heterogeneous disease. Some patients with antral dominance may even represent the ZE syndrome type I (35), without a gastrin-producing tumor but with gross hyperplasia of antral G-cells.

Duodenal Buffering and Inhibitory Mechanisms

It has often been suggested that in duodenal ulcer there is defective inhibition of gastric secretion because of the failure of the inhibitory mechanisms in either the antrum or the duodenum. Data are difficult to obtain or to interpret and do not allow any definite conclusion on this point.

However, some patients with duodenal ulcer secrete abnormally low amounts of bicarbonate into their duodenums in response to the entry of acid. The maximum secretory capacity of the pancreas, biliary tree and the duodenal mucosa is probably normal in duodenal ulcer, but these patients do not fully utilize this capacity, possibly because of impaired release of gastrointestinal hormones (such as secretin, CCK-PZ and enteroglucagon, or even GIP, VIP or motilin), which release may be depressed by extragastric vagal neural influences.

Complications of Duodenal Ulcer

Several retrospective series have shown a higher peak acid output in patients with complications such as recurrent symptoms (36), bleeding and perforation (36, 37) and pyloric stenosis (37, 38). Patients who have subsequently been operated also had higher mean peak acid outputs, even though the overlap between the groups was complete (36, 39). However, two more recent prospective consecutive series have shown no significant correlation between basal or peak acid output at the time of admission to hospital and the severity of symptoms two to four years later (40), nor were the acid secretions of patients originally selected for medical treatment, but later operated, significantly different from those of patients who continued on medical treatment (40, 41). We therefore have no clear understanding of the factors which cause the ulcer of a patient with duodenal ulcer to enter an unfavourable clinical course and to develop complications, to become intractable and to require surgery.

Gastric Secretion after Surgery for Duodenal Ulcer

All operations for duodenal ulcer aim to reduce the amount of gastric acid and pepsin flowing over the ulcer in the fasting state, after meals, during the night and indeed throughout the 24 hours. It is thus possible to discuss the rationale of the different operations for duodenal ulcer not merely empirically on the basis of the results of controlled clinical trials, but also rationally on the basis of their acid-lowering effects (42). The percentage reductions in basal and maximum acid outputs after various operations are summarized in Table IV.

The diversionary operation, gastro-jejunostomy, almost always heals a duodenal ulcer and prevents its recurrence even though about half the patients then develop a jejunal ulcer through diverted gastric secretion. It is unusual for jejunal ulcers to develop unless the preoperative gastric output was more than 30 mmol/h, possibly because the gastric secretion after gastro-jejunostomy is divided between the duodenal and stomal exits of the stomach so that neither mucosa receives the whole effect of the gastric acid. The duodenum receiving only half of less than 30 mmol/h will receive less than 15 mmol/h, the suggested preoperative threshold for duodenal ulcer, a theoretical explanation of the rarity of recurrence of duodenal ulcer after a gastro-jejunostomy.

Operations aimed at removing only parietal cells by a proximal or reverse gastrectomy or wedge resection have rarely been performed and there are few data on acid secretion at follow-up after the operations.

Antrectomy aims to remove only gastrin-producing cells and the mean percentage reduction of maximum acid output after antrectomy, 63%, is identical to the reduction after a vagotomy procedure. Thus, even though parietal cells have not been removed, their response to the maximum stimulus of histamine or to pentagastrin is irreversibly halved. The reduction in acid response to mechanical, food and vagal stimulants is not simply due to loss of gastrin re-

TABLE IV

Percentage Reductions in Basal and Maximum Acid Outputs after Various Operations
(Summarized from Tables 1, 2, 8, 10, 13 and 14 of Baron (42))

Operation	Weighted mean % Reduction in		
	Basal	Maximum	Acid outputs
Drainage alone	7	27	
"Incomplete" Truncal Vagotomy and Drainage		47	
"Complete" Truncal Vagotomy and Gastrojejunostomy	73	62	
"Complete" Truncal Vagotomy and Pyloroplasty	64	62	
Selective Vagotomy and Pyloroplasty	66	61	
Proximal Gastric Vagotomy	87	60	
Antrectomy	98	63	
Antrectomy and Truncal Vagotomy	87	89	
Billroth II Gastrectomy	87	86	
Billroth II Gastrectomy and Truncal Vagotomy		90	

lease to stimulation, because an important effect of antrectomy is a permanent reduction of the numbers of parietal cells due to removal of the trophic effect of antral gastrin on the parietal cells.

Partial gastrectomy operations aim to remove the whole antrum and a variable proportion of the parietal cells. The acid-lowering effect of a partial gastrectomy increases the higher the resection. The reduction in maximum acid output is correlated both with the actual numbers of parietal cells removed (43) and with the weight of stomach resected (44). In addition to parietal cell numbers decrease after partial gastrectomy of the Billroth II type, because of mucosal atrophy from gastritis from reflux of duodenal juice containing bile and pancreatic secretion (45).

Although a two-thirds Billroth II type gastrectomy will produce a considerable mean percentage reduction in maximum acid output of 86%, variation in acid reduction makes it impossible to predict either postoperative acid secretion or the risk of recurrent ulceration from the preoperative data (46). Recurrent ulceration after partial gastrectomy is extremely rare if the peak acid output is less than 1 mmol/h, but there is no lower limit of peak acid output (except zero) below which a recurrent ulcer can be excluded. Peak acid outputs of more than 15 mmol/h are unusual except in cases of recurrent ulcer and are present in about half of them, and peak acid outputs of more than 22 mmol/h are found only in the presence of recurrent ulcer, but only in one third of these. In clinical practice recurrent ulceration after partial gastrectomy is diagnosed by radiology and endoscopy, but an acid test showing hypersecretion may suggest not only the presence of a recurrent ulcer, but also a possible cause. Thus, a high basal output little increased by maximum stimulation might suggest an increased basal drive on the parietal cells, perhaps from excess gastrin either from a ZE tumor or a retained gastric antrum, whereas a low basal and a very high peak output might suggest a very large residual parietal cell mass for which a higher gastric resection or additional vagotomy would be needed.

It has long been a mystery why a Billroth I type gastrectomy leads to a similar percentage reduction in maximum acid output to a Billroth II type, but is followed by a much higher recurrent ulcer rate, so that this operation has been abandoned for duodenal ulcer. It is possible that food releases gastrin from the duodenum left in continuity in the Billroth I type and not from the bypassed duodenum from the Billroth II type operation (47). Unfortunately, there is no available data on the effects of either operation on duodenal acidity, or on overnight, meal-stimulated or 24-hour acidity.

Vagotomy

After truncal vagotomy all phases of gastric secretion are markedly reduced. Denervation of the parietal cells decreases their direct cholinergic stimulation as well as their indirect cholinergic potentiation of secretagogues such as gastrin. Vagotomy may impair the acid response to the distention and chemical stimulus of food in the body of the stomach by cutting the vago-vagal reflex and possibly by affecting local reflexes. Vagal denervation of the antrum reduces direct cholinergic stimulation of the G-cells as well as the indirect potentiation of the response of the G-cells to the local stimuli of food and distention. However, food-stimulated gastrin release increases after truncal vagotomy (48) possibly because of division of the extragastric vagal branches, which possibly inhibit release of extragastric gastrin, or decrease release of intestinal inhibitory hormones. The reduction in basal and maximum acid outputs shown in Table IV are similar for truncal, selective and proximal gastric vagotomy, and whether the truncal vagotomy is accompanied by gastro-jejunostomy or pyloroplasty. The "maximal" secretory response used in these calculations is the output following a conventional maximum dose of stimulus, even though it is known that higher doses of histamine or pentagastrin are needed after vagotomy to elicit the highest rate of acid secretion. A cholinergic stimulus can increase the post-vagotomy "maximal" acid output back to the preoperative acid output, so there has probably been no reduction in the number of parietal cells. Although these cells are less sensitive to pentagastrin and histamine after vagotomy, it has not been possible to restore the preoperative output, however high the dose used in man of these stimulants.

Tests of Completeness of Vagotomy

There have been several attempts to assess the degree of denervation during vagotomy operations so that if there were any nerve fibers remaining, the surgeon could cut them. The rise of gastric pressure after electrical stimulation of the vagal trunks has been measured (49), as has gastric adaptation by end-filling pressure (50), pH-mapping of the gastric mucosa after injections of histamine (51) or pentagastrin (52), and selective staining of nerve fibers with leucomethylene blue (53). These tests have not been accepted generally and surgeons still rely on postoperative gastric secretion measurements.

Methods (7, 55, 56). Basal secretion is collected as 4 × 15 min aspirates followed by a maximum stimulus of 6 μg/Kg of pentagastrin i.m. and a further 4 × 15 min (or 6 × 10 min) aspirates.

If pentagastrin is not available, tetragastrin 20 μg/Kg i.m., gastrin 2 μg/Kg i.m., Histalog 1.5–2 μg/Kg i.m. or histamine acid phosphate 40 μg/Kg s.c. (preceded by an antihistamine injection) will yield similar highest acid output from the unoperated stomach. Volume, pH and titratable acidity (pH 7–7.4) are measured. Volume × titratable acidity (mmol/1) = acid output (mmol). Acid output can be expressed as the whole hour after pentagastrin or as the sum of the two highest consecutive collection periods and this peak acid output (expressed for convenience as mmol/h) comes close to the plateau acid output after an intravenous infusion of pentagastrin 6 μg/Kg-h, an alternative, but less convenient method of measuring maximum secretion.

The Insulin Test. After the basal hour 2 ml of venous blood is withdrawn for blood glucose and 0.2 U/Kg regular insulin injected intravenously and eight more 15-minute aspirates collected with further blood samples at 30 and 45 minutes. Medical supervision is essential as well as 50% dextrose for intravenous injection if necessary. In healthy subjects (57–59) and patients with duodenal ulcer (60), single intravenous injections of insulin over the range 0.01–0.4 U/Kg gave peak acid outputs higher after 0.2 U/Kg than after smaller or greater doses, and after apparently incomplete vagotomy most patients also gave their highest peak outputs after 0.2 U/Kg (61–63). Peak acid output is correlated with the lowest blood glucose as well as the extent and rate of fall of blood glucose, so that the acid response to insulin is not an all-or-none phenomenon (57, 59). Hypoglycemia provides a quantitative glycopenic stimulus which produces a quantitative vagal secretory response (57). The optimum dose of insulin of 0.2 U/Kg in most subjects lowers blood glucose sufficiently (? to 25 mg/100 ml) to elicit a maximum vagal acid response without reducing blood glucose so low that secretion is depressed. In some normal subjects and patients after vagotomy the greatest fall in blood glucose and highest peak acid output may occur after 0.1, but not 0.2 U/Kg (63).

After the basal hour and the 2-hour collection after insulin, it is both possible and practical to perform a pentagastrin test, making a 4-hour basal-insulin-pentagastrin test (55).

An intravenous infusion of insulin 0.04 U/Kg-h (64) for 2½ hours does not reduce blood glucose as severely as a standard insulin test and has milder side effects, yet achieves comparable acidity and acid output, both in unoperated patients and after vagotomy (64–66).

2 deoxy-D glucose stimulates the lateral hypothalamus by intracellular glycopenia and may produce greater peak acid output than the insulin test (69). 2 DG has not been used widely for fear of the known and potential side effects, and it may remain a research procedure, now that there is a safe intravenous insulin infusion test.

Pathways for hypoglycemic stimulation of the stomach. Glycopenic stimulation of the hypothalamus produces both parasympathetic and sympathetic response, and hypoglycemia may also affect the gastric parietal and G-cells and the adrenals. The adrenal-sympathetic system may be a neural non-vagal pathway, but in the healthy subject insulin-stimulated secretion is unaffected by alpha and beta sympathetic receptor blockade (68). However, a beta-blocker reduced insulin-stimulated acidity and acid output after truncal vagotomy (69–71), possibly via epinephrine release of gastrin (72), so that some acid response after vagotomy may be due to sympathetic factors rather than to inadequate vagal section.

Criteria of Vagality. The Hollander (73) $+^{ve}$ criterion of an incomplete vagotomy ($\Delta[H]>20$ mmol/1, or >10 mmol/1 if $[H]_{Basal} = 0$) was qualitative and arbitrary, as are the divisions into early $+^{ve}$ (0–45 (74) or 60 minutes (75)) and late $+^{ve}$ (after 45 or 60–120 minutes) and different criteria for men and women (76). The quantitative approach uses basal and peak outputs after pentagastrin and insulin. Some users of criteria assume that an insulin test yields a $+^{ve}$ or $-^{ve}$ answer and that vagal innervation is either present or absent. A surgeon does not cut all or none of the vagal fibers to the stomach, and the higher the PAO the more parasympathetic fibers remain. This quantitative approach to vagally mediated gastric secretion is only presumptive in man but has experimental support (75–76).

Thus the problem is to compare the various measurements after vagotomies with the incidence or absence of subsequent recurrent duodeno-pyloric or gastro-jejunal ulcer, rather than with the numbers of uncut vagal fibers which are unknown. As well as using the many published criteria Kronborg (79) followed for 6–8 years 428 of 500 patients with duodenal ulcer treated by truncal vagotomy and pyloroplasty or gastrojejunostomy and used discriminative analysis to place the highest possible proportion of recurrences and non-recurrences in relation to a critical level of secretion.

This discriminative analysis was done for fasting, basal and insulin- and histamine-stimulated secretion and by comparing the 27 ulcer recurrences with both the 80 dyspeptic non-ulcer recurrences and the 321 patients free of dyspepsia (79–82). Unfortunately, these studies were done before duodenoscopy became available so that the criteria of ulcer recurrence was radiological, and actual ulcer craters were found in only 16 of the 25 patients with ulcer recurrences who were reoperated (79). The prognostic value of these calculated criteria was compared with the many older arbitrary criteria, both for tests done before vagotomy, 10 days after and 3–4 years after, since acid secretion may increase in the years after operation. Kronborg's results repay scrutiny. Here are a few examples:

Discriminant	% recurrence after 6–8 years in men with	
	>	<
Preoperative PAO_H 4.6 mmol/h	21	5
PAO_H 10 days after operation 21 mmol/h	25	5
Reduction in PAO_H 10 days after operation 68%	2	16
BAO 10 days after operation 1.3 mmol/h	22	6
PAO_I 10 days after operation 1.5 mmol/h	27	5
Hollander 10 days after operation +/−	26	7
Hollander 3–4 years after operation +/−	19	5

Thus the sensitivity and specificity of all tests are low, so that their predictive value and discriminatory ability are also poor (79–83).

A few patients after vagotomy may show a rise in *pepsin* concentration and/or output after insulin without a rise in acidity or acid output (84), but there are no other data that pepsin is a more sensitive index than acid.

The serum *gastrin* response to hypoglycemia (85) is correlated, like the acid response, with the degree of hypoglycemia evoked by intravenous injection (86) or infusion (66) of insulin. The gastrin response may depend on intact vagal fibers (87) but there have been reports of rises of serum gastrin in patients after vagotomy with no rise in acidity or acid output (88, 89). Gastrin measurements after intravenous infusion with insulin, as after single injections, do not give additional information of value in assessing vagotomy (66).

Do Tests of Completeness of Vagotomy Have Any Clinical Application?

There are four possible reasons for performing some test of vagal innervation after a vagotomy operation. The test may be done routinely after every vagotomy operation as a measure of the efficacy of the surgeon's technique, and as a means of assessing the likelihood of that patient developing recurrent ulceration. The test may be done in patients with dyspepsia after vagotomy in the hope of determining whether the dyspepsia is due to recurrent ulceration, and if so, whether there is still substantial vagal innervation which needs exploration and division.

The technique of the surgeon. There have been two studies of the results of insulin tests after operations by surgeons of different experience. Kay (90) noted that there were early positive responses in 13 out of 54 patients whose vagotomies had been performed by surgeons in training, but in none of 73 patients vagotomized by experienced surgeons. One of Kay's surgical trainees' first 24 vagotomies showed the following pattern of early positive/negative responses:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
+	+	−	−	+	+	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−

Johnston and Goligher (91) on the other hand, found the mean percentage of incomplete truncal vagotomies by 9 consultant surgeons (18%) to be higher than those by 15 senior registrars and registrars (12%) even though the range was smaller in consultants (8 to 45%) than in their assistants (0 to 52%). There were similar results after selective vagotomy, but there was a marked decrease over the years 1966–1970 in the incidence of positive Hollander responses after selective vagotomy. Unfortunately, there are no available data on the efficiency of different surgeons in reducing acid output after vagotomy operations. Acid tests are, therefore, of some value to the efficiency of the surgical team in assessing the completeness of each vagotomy.

The Prognosis for the Patient. Although the various tests described above do indicate the relative risk of recurrent ulcer, the overlap between the groups is almost complete and it is impossible in an individual patient to predict reliably whether recurrent ulceration will develop, and whether any patient would therefore need a more careful follow-up. For this reason it seems unnecessary to perform acid tests routinely after operation, merely for the sake of predicting those patients with a greater chance of recurrence. There are other reasons why patients after vagotomy operations should be followed up regularly. Patients soon tell their doctors if their dyspepsia has returned and those patients with recurrent symptoms can then be endoscoped. Patients with dyspepsia after vagotomy are more likely to have recurrent ulcers, not only if they have positive Hollander tests or higher peak acid outputs after insulin, but also if they have higher basal or "maximal" pentagastrin-stimulated acid outputs.

Absolute anacidity excludes a diagnosis of recurrent ulceration. A low basal, with a low insulin-stimulated, but high pentagastrin-stimulated, acid output suggests that the patient has a very large functional parietal cell mass, even if there is no obvious vagal or gastrin drive.

A high basal secretion with a positive Hollander test and a high peak acid output after insulin, especially in relation to the pentagastrin-stimulated acid output suggests not only that recurrent ulceration is likely, but that it was associated with significant remaining vagal innervation.

A high basal secretion with a low peak acid output after insulin, especially in relation to the pentagastrin-stimulated acid output, suggest that any recurrent ulceration is unlikely to be due to inadequate vagotomy, but is probably due to excess antral or extragastric gastrin secretion which needs appropriate investigation. If, however, the primary surgical treatment for recurrent ulcers after vagotomy (except for ZE) is antrectomy and re-vagotomy is of secondary importance, then the value of acid tests in assessing vagal innervation is open to question.

Conclusion. After vagal denervation routine acid tests are probably useful as part of the systematic assessment of the vagotomy and the vagotomist, and possibly useful in symptomatic patients in the evaluation of residual innervation in planning revision surgery.

Rationale of Vagotomy in Duodenal Ulcer

Even after 'incomplete' Hollander-positive vagotomies the mean reduction in maximum acid output of 47% was three quarters of the reduction of the 62% found after "complete" Hollander-negative vagotomies (Table IV). For the sake of discussion the threshold acid output for recurrence of duodenal ulcer after vagotomy or pyloroplasty will be assumed to be the same as the preoperative threshold of 15 mmol/h peak acid output, or 11–12 mmol/h maximum acid output. Then even after an "incomplete" vagotomy with a 47% mean reduction, acid outputs of less than 30 mmol/h will be reduced below this threshold of 15 mmol/h, whereas peak acid outputs of as high as 40 mmol/h will be reduced to below this 15 mmol/h threshold by "complete" vagotomies (with a 62% reduction). Alternatively, one can allow for the drainage procedure. Post-vagotomy plateau acid output mmol/h = 0.64 pre-vagotomy output—9.6 (96); and once again patients with acid outputs of less than 40 mmol/h will be reduced to 15 mmol/h by "complete" vagotomy. Pre- and post-vagotomy acid secretion are correlated, but there is unfortunately a wide scatter around the regression line, so that one cannot accurately predict the post-vagotomy acid secretion of a particular individual, even with the help of a "medical" vagotomy. The general hypothesis can be tested by considering the risk of recurrent ulceration after vagotomy according to the pre- and postoperative maximum acid output. In three small British series (83, 93, 94) recurrent ulceration was equally common in lower and upper halves of the range of acid secretion before operation in duodenal ulcer. However, it will be seen from Figure 1 that in the large Copenhagen series, recurrent ulceration was twelve times as common in those who before operation were in the upper range of peak acid output. The

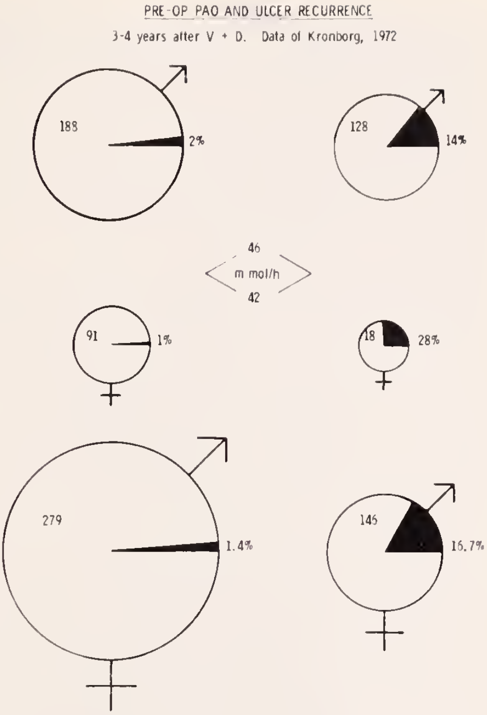

FIG. 1. Peak acid outputs of patients with duodenal ulcer before truncal vagotomy and pyloroplasy/gastro-jejunostomy, and the incidence of ulcer recurrence 3–4 years later (Data of Kronborg (80)). The areas of the circles are proportional to the numbers of male, female and all patients in each category of acid output and the black segments are proportional to the percentage recurrences shown.

absolute reductions of peak acid output after vagotomy in patients with and without recurrence were similar. Patients who after vagotomy were left with peak acid outputs of above 20 mmol/h had six times the incidence of recurrent ulcer. These high post-vagotomy acid outputs in patients with recurrent ulcer were not due to these patients having smaller absolute reductions of peak acid output because these reductions were similar in patients with and without recurrence. They were due to a percentage reduction in acid output after vagotomy of less than 70% being eight times more common in patients with, than those without, recurrent ulceration. Thus Kronborg's 3–4 year follow-up data (80), which have been borne out by a recent 6- to 8-year follow-up of the same patients (82) support the hypothesis that patients with high preoperative peak acid outputs are less likely to be reduced below an absolutely or relatively safe threshold, and therefore have a higher incidence of recurrent ulcer.

Selective Vagotomy. The percentage reductions in secretion after selective vagotomy and drainage were almost identical with those after truncal vagotomy (Table IV), so that the rationale of this operation must be primarily based on clinical evidence of decreased incidence of sequelae.

Proximal Gastric Vagotomy. The percentage reductions in acid secretion soon after this operation with or without drainage are similar to those after the older vagotomies (Table IV), so that again the rationale of this latest operation must be based on clinical evidence of follow-up studies.

Vagotomy and Antrectomy. An antrum which has been denervated by truncal or selective vagotomy can still release gastrin and an antrectomy performed after truncal vagotomy produces a further reduction in maximum acid output from 62% to 84% (95). It can be seen from Table IV that vagotomy and antrectomy is a most effective acid-lowering operation, which would theoretically on average reduce peak acid outputs of up to 60 mmol/h down to the 15 mmol/h threshold and thus explain the very low recurrence rate after this operation.

Selective Surgery. If a gastric surgeon aims to reduce gastric acid below a threshold figure, and if acid-lowering operations tend to reduce acid secretion by a fixed amount or a fixed percentage, then patients with higher acid outputs should require a more acid-lowering operation than those with lower acid outputs. Selective surgery for duodenal ulcer was introduced by Daintree Johnson (96) and at that time he advocated partial gastrectomy for most patients, with an additional vagotomy for those with extreme hypersecretion. At least 13 such schemes have been published in which various radical operations have been used for patients with higher acid outputs (55). Almost half the British gastroenterological surgeons surveyed (54) were influenced by the results of acid tests in deciding the type of operation they used in chronic duodenal ulcer, most performing a vagotomy in patients with low peak acid outputs, and vagotomy and antrectomy or partial gastrectomy in those with high peak acid outputs: some used only gastro-jejunostomy when peak outputs were less than 25–30 mmol/h. The absence of controlled trials and the paucity of convincing follow-up studies make it difficult to assess the usefulness of selective surgery. The Copenhageen data (80) suggest that recurrent ulceration occurs at an unacceptably high rate after vagotomy in that a third of patients with duodenal ulcer with a preoperative peak acid output of more than 46 mmol/h, and it is probably that these patients would have avoided recurrent ulceration if they had been treated by vagotomy and antrectomy at the first operation. Recurrent ulcer is not, however, the only problem of surgery for duodenal ulcer. The more radical the acid-lowering operation, the more likely is the mortality and morbidity to rise as well. About 30,000 British patients are operated each year for peptic ulcer. The first column in Table V estimates what would happen in a seven-year follow-up if this number of patients with duodenal ulcer were all operated by vagotomy irrespective of their peak acid output, assuming a 0.8% mortality for vagotomy (97), a 10% recurrent ulcer rate (82), and a mortality for reoperation of 2%. The other two columns estimate the fate of this same group of patients if that third with a peak acid output above 46 mmol/h were selected for vagotomy plus resection, with a doubled operative mortality of 1.6% (97), and a recurrent ulcer rate of only 0.5%, instead of 5% for the two thirds of those patients with duodenal ulcer with a peak acid output less than 46 mmol/h and treated by vagotomy alone. It can be calculated that the increased mortality from the increased recurrence and reoperation rate of patients having a vagotomy as a primary operation is overweighed by the

TABLE V

Elective Surgery for Duodenal Ulcer—Balance Sheet at 7 Years

	All Patients Any PAO	Patients Divided by PAO	
		2/3 <46 mmol/h	1/3 >46
	Vagotomy No resection	Vagotomy No resection	Vagotomy + resection
	30,000	20,000	10,000
Mortality	0.8%	0.8%	1.6%
Deaths	240	160	160
Leaving	29,760	19,840	9,840
Recurrent Ulcer	10%	5%	0.5%
Reoperated	2,976	992	49
Mortality	2%	2%	2%
Deaths	60	20	1
Total 7-year deaths	300	180	161
		341	

increased mortality of the primary more radical acid-lowering resection operation, although admittedly no allowance has been made in this model for the morbidity and complications of the thousands more patients developing recurrent ulcer if vagotomy was the primary operation for every duodenal ulcer.

Conclusion

This review has been limited to a consideration of the etiology of duodenal ulcer disease as part of a hypersecretory diathesis, and of operations for duodenal ulcer on the basis of their acid-lowering effects.

There are data which provide a rationale for selective surgery for duodenal ulcer, but published results have not yet established the value of this policy, nor have there been any prospective randomized trials. On the available evidence, a vagotomy procedure may be suitable operation for all patients with duodenal ulcer (except ZE), whatever their acid status, but it is possible that a more radical procedure may be shown to be indicated for a proportion of patients with duodenal ulcer with marked hypersecretion.

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The Impact of Controlled Trials on the Practice of Medicine*

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Abstract

Professional Standards Review Organizations will presumably employ peer review committees to set standards. It is unclear whether such committees will rely more on the results of clinical trials or on the "usual practice of the community." For this study several techniques have been employed to assess the use by physicians in practice of therapies whose efficacy has been brought into question by clinical trials. The sales of oral hypoglycemic agents have continued to rise since 1970 in spite of the suggestion in the UGDP studies that they cause increased deaths from cardiovascular disease. For 15 years physicians continued to prescribe stilbestrol for roughly 50,000 pregnant women a year in spite of the fact that six reasonably well-controlled studies in the early 1950's showed it to be totally ineffective. A review of hospital records reveals that strict bed rest continues to be prescribed for patients with acute viral hepatitis, and the Sippy and related bland diets for patients with peptic ulcer, in spite of the fact that all controlled trials conducted have shown both therapies to be worthless. It is suggested that physicians who wish to continue to employ therapies that have been found by peer review to be ineffective, or more toxic than efficacious, should be expected, as in the case of investigative procedures and drugs, to submit their plans to peer review and to obtain truly informed consent from their patients.

The success of professional standards review organizations will depend on physicians' agreeing on definitions of high quality in patient care. It has been traditional to define good medical care, at least within the legal sense, as 'that care ordinarily practiced by physicians in the community.' A more effective basis for setting standards would be the conclusions of well-conducted clinical trials. Hopefully, review boards will depend on the interpretation of these by experts and will base their prescribed standards on these interpretations. There is little information in the literature on the degree to which the practice of medicine in the community is a reflection of the results of controlled clinical trials. In order to obtain some data on this important relationship, four areas of therapeutic endeavor have been reviewed.

The University Group Diabetes Program reported in 1970 (1) the results of a long-term comparison of tolbutamide and phenformin with a placebo and with insulin in patients with adult onset, non-insulin-dependent diabetes. Although the study was originally designed to determine whether or not therapy would prolong the life of diabetics by diminishing vascular disease, it had to be stopped prematurely because both the oral agents were associated with a statistically significant increase in mortality from cardiovascular disorders. Because the result was so contrary to what most physicians expected and hoped of the drugs, there has been a continuing battle in the literature on the validity of the UGDP

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study. Much smaller trials in patients with much less hyperglycemia by Keen and Jarrett (2) and by Paasikivi (3) have been cited as conflicting evidence. The debate will not be settled until a blue-ribbon committee appointed by the Biometric Society reports its evaluation of the statistical validity of the three studies. In the meantime two studies (4, 5) of what happens to patients when they are taken off oral agents because of the possibility of increased risk have revealed that from one-third to two-thirds did not need to be on the drugs at all. One might expect that because of the possibility of harm by agents not required in a large percentage of the patients in whom they had been used, there might be less use of the drugs. The opposite is true, as revealed by Table I. Mentions of oral agents by physicians in practice have continued to increase since 1970.

In the last few years many cases of adenosis of the vagina and a few examples of carcinoma have occurred in young women whose mothers had received stilbestrol to prevent spontaneous abortion (6). In 1971 the Food and Drug Administration issued a directive against the use of the drug for this purpose (7). Obstetricians cannot be faulted for not anticipating such a long-term side-effect of the therapy. At the time of its use there was no known toxicity, but was there any evidence of efficacy? Between 1946 and 1955 thirteen evaluations (8) of the efficacy of stilbestrol in preventing abortions in women with histories of habitual abortion and in diabetic women were carried out. Seven of the studies (9–15) resulted in enthusiastic conclusions (Table II). None of these seven were controlled trials. Three had no controls, two employed historical controls, and in two studies the controls can at best be called contrived, because the data were gathered after the results had originally been reported without any control patients. During this same period of time six studies (16–21) (Table III) revealed no evidence for efficacy for stilbestrol. All six of these had simultaneous control patients and three were double blind. In the early 1950's randomization was not a commonly used procedure, and only one of the six was a randomized controlled trial.

These data should have been most impressive to practicing physicians. The evidence was overwhelming that stilbestrol had no effect in preventing spontaneous abortion in any group of patients, and six of seven textbooks of obstetrics

TABLE I

Oral Hypoglycemic Mentions by Private Practitioners in the United States

1967	15,015*
1968	16,967
1969	17,471
1970	17,676
1971	17,168
1972	18,486
1973	19,545

* × 1,000.

Data from the National Disease and Therapeutic Index, Ambler, Pa.

TABLE II

Diethylstilbestrol in Prevention of Abortion and Other Accidents of Pregnancy

Author	Enthusiastic Studies			Blinding
	Date	No. of Patients	Controls	
Smith (9)	1946–1954	1300+	Contrived	No
Gitman (10)	1950	51	None	No
Davis (11)	1950	42	Historical	No
Ross (12)	1951–1953	200	None	No
Pena (13)	1954	200	Historical	No
White* (14)	1949–1953	642	Contrived	No
Plate (15)	1954	29	None	No

* Diabetics.

TABLE III

Diethylstilbestrol in Prevention of Abortion and Other Accidents of Pregnancy

First Author	Negative Studies			Blinding
	Date	No. of Patients	Controls	
1. Crowder (16)	1950	100	Alternate	No
2. Robinson (17)	1952	93	Alternate	No
3. Ferguson (18)	1953	393	Alternate	Yes
4. Dieckmann (19)	1953	1,646	Alternate	Yes
5. Randall (20)	1955	237	Simultaneous	No
6. Reid* (21)	1955	148	Random	Yes

* Diabetics.

came to this conclusion during the 1960's. Yet several studies of the marketing of stilbestrol in the late 1960's revealed (22) that roughly 50,000 women received the drug during pregnancy per year, 15 or more years after six reasonably well-controlled studies showed it to be totally ineffective. The late-appearing toxicity was irrelevant.

After World War II it was common practice to require patients with acute viral hepatitis to stay in bed until their liver function tests became normal. This was based on clinical observations of the patient as his own control, or on studies carried out in World War II in which the course of patients treated with strict bed rest and high protein diet in an Army Hospital in Rome was compared with that of a previous group of patients treated with ad lib rest and an ad lib diet in Naples (23). Two trials of bed rest with simultaneous controls carried out during and after the Korean War have revealed absolutely no suggestion of a therapeutic benefit (24, 25). To ascertain the effects of these controlled trials on the practice of medicine, records were reviewed in two community and two university hospitals. The orders written on admission of patients with the diagnosis of viral hepatitis were divided into two groups: 1) strict, modified, or

TABLE IV

Admission Orders Written for Patients with Acute Viral Hepatitis

	Strict or Modified Bed Rest	Ad Lib or No Mention of Bed Rest	Total
Two University Hospitals	58 (49%)	60 (51%)	118
Two Community Hospitals	48 (67%)	24 (33%)	72
Total	106 (56%)	84 (44%)	190

bed rest with bathroom privileges; and 2) ad lib rest, or no mention of rest in the admission orders (Table IV). No mention of rest in the admission orders (Table IV). Ten to fifteen years after the first definitive study showed that it was a totally ineffective therapy, doctors were still keeping 67% of the community hospital patients and 49% of the university hospital patients in bed after admission to the hospital for acute hepatitis.

There have been eight studies of the composition of the diet in the treatment of acute peptic ulcer (26–33). All have shown that healing is not hastened by administration of a Sippy diet or a bland diet. Donaldson has clearly reviewed the infrequency of the need for specific diets in the treatment of peptic ulcer (34). Yet in a community hospital, 15 of 16 physicians ordered such a modified diet and 20 of 22 did so in a university hospital. Fifteen of these 38 physicians ordered a Sippy diet. Again, there is not much evidence that controlled trials are influencing the practice of medicine.

There are many possible explanations why in these four instances physicians do not seem to be paying much attention to the literature. It is possible that some are not well enough educated in the fine points of controlled trials, and are influenced by the bad studies as well as the good. In some cases the practicing physicians may not have read any of the original trials but may rely on reports in the many "throw-away" journals designed to save time for busy practitioners. In the case of the oral hypoglycemic agents the throw-away journals have been blatantly biased in favor of the drugs.

Advertising by pharmaceutical houses does not explain why physicians hang onto the formally traditional therapy of bed rest for hepatitis and a Sippy diet for peptic ulcer. Here innate conservatism may play a role, or possibly the need for the physician to order some form of therapy for his patient, whether or not there is any evidence that it works. The "it can't do any harm" philosophy has had a powerful influence on the practice of medicine. The late-appearing toxicity of stilbestrol and the oral hypoglycemic agents emphasizes the fallacy of this reasoning.

At any rate, these data illustrate a clear-cut dichotomy between the "standards of the community" and the scientific data obtained from clinical trials. Presumably peer review committees will recognize the latter as more reliable.

What is to protect a patient from the prescription by his physician of an ineffective medication or therapy, or one characterized by a clearly negative benefit-to-risk ratio? Food and drug laws require proof of efficacy for new drugs, but the Food and Drug Administration has been slow in ordering the withdrawal of drugs found by peer review committees to be ineffective. There are no laws covering therapies such as bed rest, diet or operative procedures, new and old. At the present time patients are protected from inappropriate trials of new therapies by the food and drug laws and by regulations of the National Institutes of Health requiring peer review and the obtaining of truly informed consent from the participant in any research project. Although the rules do not apply to research that does not involve new drugs and is not supported by the National Institutes of Health, broadened regulations and legislation now being considered by Congress may fill those loopholes.

There is a distinct disadvantage, however, in regulating only the trials of new therapies, for the added administrative details and elaborate informed consent procedures may be expected to discourage physicians from embarking on trials of new agents. The scales will be tipped towards the status quo, and progress in therapy will be distinctly hampered. However, that would not be the case if physicians accepted the principle that those who wish to continue to use old therapies that have been shown by clinical trials to be ineffective or hazardous should also be required to prepare a protocol defending their desire to continue the therapy in specific instances because they do not believe the pertinent clinical trials. It is important that physicians have that right, but it is just as important that patients be protected from the indiscriminate use of ineffective medications as from the indiscriminate trials of new agents.

The principle should also apply to informed consent. It is now required that physicians undertaking experimental therapies obtain informed consent by methods that are adequate and appropriate. The basic elements of such consent have been summarized as follows (35):

1. A fair explanation of the procedures to be followed, including an identification of those which are experimental;
2. A description of the attendant discomforts and risks;
3. A description of the benefits to be expected;
4. A disclosure of appropriate alternative procedures that would be advantageous for the subject;
5. An offer to answer any inquiries concerning the procedures;
6. An instruction that the subject is free to withdraw his consent and to discontinue participation in the project or activity at any time.

Why should not patients who are asked by their physicians to be treated according to a regimen that has been discredited by controlled trials be offered the same protection? If a peer review group should find that oral hypoglycemic agents should not be used in adult onset diabetes unless the patient's symptoms are not controlled by diet and/or insulin, or if the patient is considered unable to take insulin, the informed consent procedure should include notification of the patient of that fact and the reasons why the physician wishes to use the agent anyway. Stilbestrol may no longer present a problem, since its use in pregnancy has finally been banned by the Food and Drug Administration. It seems likely that a peer review group would declare bed rest unnecessary in acute viral hepatitis and a Sippy diet uncalled for in patients with ulcer. If so, it is not unreasonable to require that doctors who wish to continue to use these therapies should prepare a protocol for approval by their peers and obtain the informed consent of their patients.

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Transplantation Biology: An Appraisal

SIR PETER MEDAWAR*

When Dr. Sol Berson came to spend a few months at the National Institute for Medical Research in London in 1967, his many English admirers and friends hoped that he would regard this period as time out for relaxation and reflection —perhaps even as a holiday; but those who knew him better than we had yet had an opportunity to, would have realized that any such hope was vain, for it was not in Sol Berson's constitution to take things easily. He set us, instead, an awe-inspiring example of single-minded dedication to the pursuit of knowledge.

In spite of the distinction of his own necessarily specialized researches—in real life none of us can work on more than a small part of the canvas—Berson was one of those rare people who would always see the whole picture. Indeed, his grasp of everything cognate with his chosen subject was quite remarkable. For this reason I make no apology for commemorating him in the form of a rather general lecture about immunological ideas rather than in a lecture dealing with particular specialized researches.

Over the past five or ten years many hospitals and research institutes have been asking themselves about the advisability of setting up new divisions or sections devoted to a science unheard of as recently as thirty years ago: transplantation biology. Many such institutions took the plunge, so now is a good time to ask if their action was well advised, and whether transplantation biology has indeed achieved what its more sanguine practitioners expected of it and told its sponsors it would certainly achieve.

I should like, however, to begin with a number of general remarks about the history of ideas as exemplified by the recent growth of immunology. Everybody knows that transplantation biology has made a most important contribution to that tremendous efflorescence of immunology which has been the most prominent feature of the growth of the medical sciences since the war. What transplantation biology did was, in effect, to liberate immunology from the hegemony of bacteriology. For very many years immunology was merely part of the small print in the syllabus of bacteriology courses; it was a matter of vaccines, antisera, skin tests, agglutination reactions, complement, and not very much else. It was, in fact, a typical applied science, energized by nothing except the promise of practical rewards. As a young research worker I can remember immunology's being jeered at in a genteel sort of way by biochemists and biophysicists for its barefaced empiricisms and the almost idiotically simplistic terminology that seemed to go naturally with them. The lag phase in the growth of immunology ended when immunogeneticists, immunochemists and immunozoologists altered its entire center of gravity to the study of the biology of self-recognition, the molecular basis of specificity, and to the nature of information transfer in biological systems—still the energizing concepts of immunology.

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* This, the Second Solomon Berson Memorial Lecture, delivered by Sir Peter Medawar, distinguished immunologist and Nobel Laureate, honors one of the brightest luminaries in the galaxy of great minds and great men in the more than one hundred years of Mount Sinai history. *Editor's note*

I have not yet said what I conceive 'transplantation biology' to be, and I take it to be not just the theory and practice of organ and cellular transplantation, but to comprise all branches of immunobiology that have been inspired by or made possible by the techniques of transplantation. However, it would be merely perverse not to put transplantation first among the benefactions of transplantation biology. It was not so long ago that, as a deterrent to undue enthusiasm, transplantation surgeons spoke gravely of transplantation's being an experimental procedure, justified only by the imperative need to save life—an appeal always upheld in the court of clinical judgment. Today, however, kidney transplantation has been received into the ordinary repertoire of surgical practice in all leading medical centers throughout the world. Heart transplantation and lung transplantation will come too. Anybody who declares they will not, is making a prediction of just the kind that science most often and most easily falsifies. They will come if they are wanted and if their coming is not impeded by non-medical considerations. The encouraging thing about heart transplantation is that the results are steadily improving. There is room for improvement in kidney transplantation, too, for no one will be satisfied until quantitatively and qualitatively the life expectancy of the transplant patient is shown to be not less than that of normal human beings of the same age and sex.

Turning now to general biological and immunological contributions of transplantation biology, I propose to give pride of place to the discovery of the immunological functions of the thymus. All those ostensibly simple-minded teleologists who strongly suspected that such a large and important-looking organ must surely have some function in the body have been triumphantly vindicated by the work of Robert Good, Jacques Miller and their respective associates. With this discovery went the recognition of a categorical distinction between humoral immunity and cell-mediated immunity, a distinction that was first made operatively in the study of transplantation and other delayed-type sensitivity reactions, and now put on a firm foundation by the recognition of the distinction between thymus-dependent cells, and cells which in birds and reptiles depend on the Bursa of fabricius. In mammals this class of cells depends on whatever organ is the homologue of the Bursa—and for that reason they are universally known as 'B-cells', a term that is likely to stick even if the homologue is discovered and turns out not to begin with a 'B.'

The next concept derived from transplantation biology that deserves special mention is that of immunological tolerance. The first example of the phenomenon we now call tolerance or paralysis was the non-reactivity of mice toward relatively high doses of an antigen—pneumococcal polysaccharide—which in lower doses would have excited an immune response. But even before Felton's discovery, as Mitchison has pointed out, a rabbit featured in a paper written by A. T. Glenny behaved in a way that can now only be interpreted in terms of tolerance. I think of this rabbit as an actor whose name once fleetingly appeared in the cast list of an old silent movie.

It was, however, in the context of transplantation that the notion of tolerance rose to such prominence, and here perhaps it should be pointed out that, contrary to a widespread belief, there was no direct connection between the discovery of tolerance and the demonstration that transplantation was clinically feasible. The importance of the discovery was almost wholly a moral one: it showed that the transplantation problem was a soluble one; until it was made, it would have been perfectly possible for someone with a despondent turn of mind to say that the transplantation problem could not be solved. Transplantation antigens were genetically programmed and to change them would be a feat of the same magnitude as to change somebody's blood group; moreover, there seemed no reason to believe that the transplant rejection reaction could be circumvented or annulled. The very earliest research on tolerance showed that it could be so and this aroused world-wide interest in the possibility of using transplants in clinical practice.

We all know that the use of transplants in clinical practice is founded squarely upon the use of immunosuppressive agents and it is not unreasonable to say that nearly all modern research into immunosuppression has derived its inspiration from transplantation biology. It began with the tentative exploratory trial of the antiproliferative drugs used in cancer chemotherapy—e.g., nitrogen mustards, antimetabolites and DNA-base analogues—but has extended since to exploring the preparation and properties of acute lymphocytic globulin. Correspondingly, the uses of immunosuppressive agents extend beyond transplantation into the area of autoimmune diseases and diseases with an autoimmune component, including some of the neurological diseases. Here I think it must be said that by contrast with what so often happens, European research has been more ambitious than American research in exploring this general area.

Under the heading of tolerance it is natural to include the interpretation of the recovery from radiation injury made possible by injecting spleen extracts. It was soon discovered by the application of transplantation techniques that the restorative process had, in effect, the character of the transplantation of blood-forming cells.

The study of recovery from radiation injury by treatment with allogeneic bone marrow soon led to the discovery that mice spared rapid death from acute radiation injury were often spared merely to undergo a lingering death as a consequence of what came to be called 'secondary irradiation disease.'

The interpretation of this disease had to wait upon the discovery of a new modality of immune response by M. Simonsen, and by R. E. Billingham and L. Brent. This was, of course, the 'graft-versus-host reaction,' the importance of which is twofold: partly as a dire warning of the dangers that may attend the transplantation of lymphoid cells and partly as it made possible an assay of immunological competence, especially in the classic experiments of J. L. Gowans. It also provides one of several possible models of autoimmune disease.

The induction of tolerance is not the only stratagem at our disposal for establishing a specific non-reactivity towards transplantation antigens, for another and entirely different approach devised by Snell and Kaliss has grown directly out of transplantation biology: the method of 'enhancement,' by which I understand all systems of immunosuppression that depend essentially upon the use of the immunosuppressive properties of specific humoral antibody. Under this general heading I also include modern attempts to devise theories of immunoregulation based on the one hand upon antigenic drive and on the other upon inhibitory feedback by antibody.

It may be that in the practice of transplantation 'enhancement' methods will eventually come to supplant immunosuppressive methods founded upon the rationale of tolerance, by which I understand the combination of high antigen doses with nonspecific immunosuppression.

Nobody with any understanding of the growth of scientific ideas will for one moment question the proposition that modern tumor biology is an offshoot of transplantation biology. Tumor immunology began with the discovery that autochthonous and syngeneic tumors can excite an immunological reaction in the animals that bear them. There is much in common between the rejection of a tumor and the rejection of an allograft but it would be quite unduly simplistic to say that the processes were identical. Mechanisms other than straightforward T-cell-mediated immunity are responsible for checking the growth of tumors and I do not think a rational tumor therapy can be devised until we have learned what these ancillary mechanisms are. Just as the study of immunosuppression grew out of tolerance, so now the much more fashionable study of immunopotentiation has grown out of tumor biology.

The record of achievement I have outlined above is a pretty impressive one, so all those institutions that decided after more or less hesitation to establish departments or sections of transplantation biology are to be congratulated on their prescience; conversely, those who did not deserve all the odium of public censure.

What more general lessons can be drawn? Important though they are, or promise to be, not one of the advances I have outlined has grown immediately out of a direct attempt to solve a practical problem. They have been the product of the ordinary scientific process of which the driving force is that hunger for understanding which is the mark of the true scientist as opposed to the man who is primarily in science to make a living.

In the advancement of science narrowly and perhaps selfishly considered, the importance of the practical goal is in the first place to secure the funding of the research enterprise and in the second place to inspirit and reassure the research worker who asks himself from time to time whether or not he is doing something really useful to someone other than himself. I believe that all good research workers put such questions to themselves and any misgivings they may have are most easily allayed by the conviction that their work may have a manifestly useful practical outcome. Another important consideration is that it supplies the research worker with a livelihood, for no one is going to subsidize a lifelong's intellectual recreation.

Sol Berson was a man inclined to philosophic discussion, and during his brief period in London I had many discussions with him in the general style of that which I have just been conducting with myself. I am happy and relieved to say that I don't think Sol Berson would have disagreed with anything I have said, though he might have expressed much of it a good deal more forcibly than I have done.

I have said that the accomplishments of transplantation biology make an impressive story, but the story is not yet half told. Among the future developments we may look forward to are: the completion of the functional taxonomy of lymphoid cells, amounting almost to an ethnology of lymphoid cells (a process of which we see the mere beginnings in the subdivision into T cells and B cells); next, we look forward to the identification of all differentiation antigens and organ-specific antigens that can act as alloantigens (for example, the skin-specific alloantigen of Lance and Boyse); then again, we must hope for a complete mastery of immunoregulation, not just of immunosuppression and immunopotentiation, although these are, of course, vitally important elements of immunological control.

The practical rewards that may be expected to follow from these fundamental advances are indeed enormous; among them are the transplantation of heart, lung, bone marrow and perhaps even of skin, the control of autoimmune diseases and of all diseases that have an autoimmune component in their natural history. These are great rewards and the task of achieving them will be proportionately difficult. Today we must regret with special poignancy that Solomon Berson is no longer here to help us with their solution.

The Present State of Toxoplasmosis

TIBOR SZABO, D.V.M.

Introduction

Toxoplasma gondii (T. gondii) was first recognized in acute infections of gondis (rodents) and rabbits. The name was derived from the Greek word 'toxon,' meaning bow or arc, a reference alluding to their curved lunate shape. They are not toxic; however, their harmful effects depend mainly on intracellular parasitism.

Distribution of T. gondii is unusual in that it is found in most parts of the world and has little host specificity. It can infect any warm-blooded animal, including birds. The prevalence of infection in man, as judged by antibody tests, varies from as low as 4% in Navajo Indians to 94% in Guatemalans. In England the prevalence among adults varies from area to area, but on the average is about 30% (2). More than a third of the population in most parts of the world have antibodies indicating past infection with T. gondii (3, 4). Although toxoplasmosis is not a common clinical entity, it continues to be a significant public health problem in terms of severity of disease (5). This is confirmed by recent reports of toxoplasmosis in human fetuses and neonates (6–9), children (10–12), adults (13), cancer patients, transplant and transfusion patients (14), and ophthalmic patients (15). Corresponding disease also continues to occur in pets (10), livestock (16), laboratory animals (17), and wildlife (18).

In Paris, nearly 100% of the adult female population have had immunologic experience with toxoplasma by their twenties. In the United States, a study among armed services recruits undertaken in 1969 showed a remarkably wide range of positive reactors depending on the part of the country from which the young men came. Only 3% of soldiers from the Rocky Mountain states had antibodies, a prevalence rate of about one-seventh of that found among men from the North East. Pacific Coast recruits were intermediate with a prevalence rate of 8% (19). There is no scientific explanation existing regarding the regional differences (Fig. 1). However, there is no doubt that this uncertainty is responsible for the inability to determine how the organism is transmitted in nature.

Present Life Cycle

T. gondii is a coccidian parasite and like most other coccidia it has an enteroepithelial cycle in a specific host, resulting in the formation of oocysts. Only domestic cats and certain other members of the felidae have been shown to produce Toxoplasma oocysts (3). The nomenclature for the asexual and sexual stage of the enteroepithelial cycle of T. gondii is typical of other coccidia. Atypically, however, some of these enteric stages of Toxoplasma organisms pass

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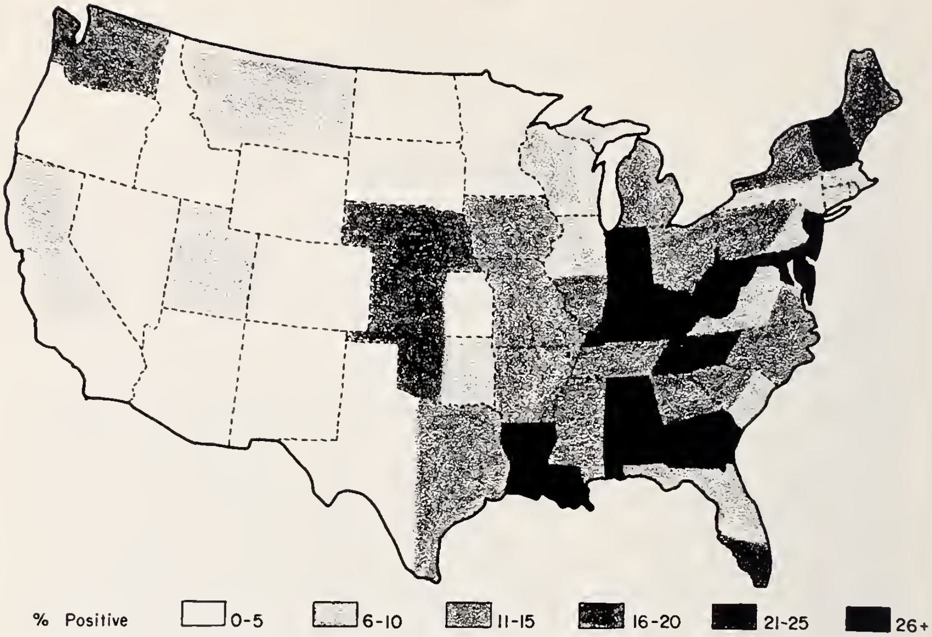


FIG. 1. Prevalence of Toxoplasma infection in the United States.

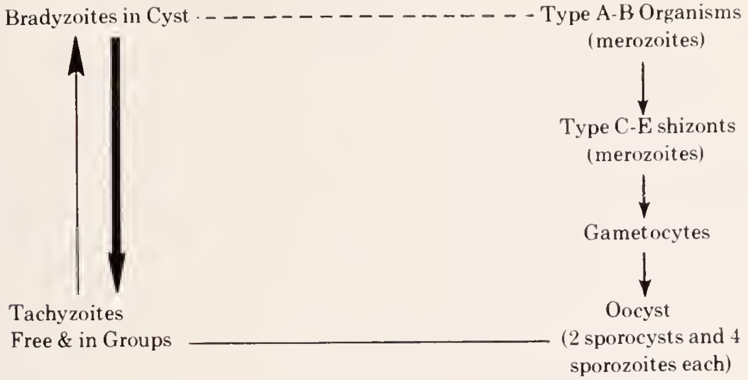
through several multiplicative generations and have been designated types A–E (3–20).

Unlike most coccidia, T. gondii has an extracellular (tissue) cycle. At least two old and two new systems of nomenclature exist at the present time for these asexual extraintestinal stages (3–21). In the past the individual organisms in tissues were called 'merozoites,' cyst forms, or just 'zoites.' The new terms suggested are 'bradyzoites,' reflecting the slow replication of encysted organisms, or 'cystozoites' (21), indicating their occurrence in cysts. There is confusion and controversy (3, 22, 23) over the misleading and possibly inappropriate term 'pseudocyst' which has been used both for cysts and noncysts. 'Bradyzoites' thus appears to be a more meaningful term than cystozoites. The terms 'trophozoite' or 'proliferative form' have been used for individual organisms found free or intracellularly in tissue groups. The new terms suggested for trophozoites are 'tachyzoites,' indicating the rapid replication of this stage, or 'endozoites,' reflecting replication by endodyogeny (division into two internal buddings). Endogeny also occurs in bradyzoites and type A and B organisms, however, and endopolygeny (multiple endodyogeny) occurs in type B and A organisms. Therefore, the term tachyzoites seems preferable to endozoite. In addition, tachyzoites correlate with bradyzoites in indicating the rate of replication of these organisms (3).

Schematically (Table I), a heavy arrow is used in the direction from tachyzoites to bradyzoites to indicate the usual direction of development (3). These various stages and types of Toxoplasma organisms have been summarized or

TABLE I

The New Nomenclature for the Various Forms of Toxoplasma Organism


Bradyzoites in Cyst
Type A-B Organisms (merozoites)
Type C-E shizonts (merozoites)
Gametocytes
Tachyzoites Free & in Groups
Oocyst (2 sporocysts and 4 sporozoites each)

grouped into five major stages (3). Three of these stages occur only in the feline enteroepithelial cycle.

1. Enteroepithelial multiplicative stage
2. Gametocyte stage
3. Oocyte stage

The fourth and fifth stages occur in the extraintestinal (tissue) cycle in incomplete (intermediate) as well as feline host.

4. Tissue group stage of tachyzoites formed during acute infection.
5. Tissue cyst stage of bradyzoites formed during chronic infection.

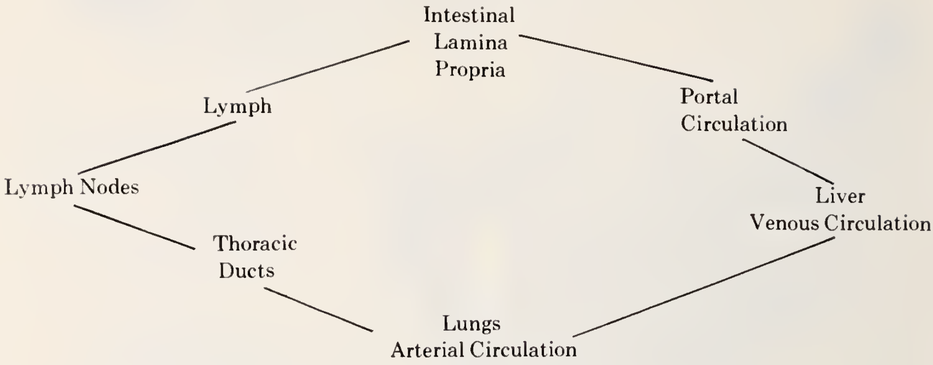
Various investigations are under way presently to determine the prepatent periods (time elapsed until oocysts appear in feline feces) after ingestion of tachyzoites, bradyzoites or oocysts (4).

Pathogenesis

After ingestion of encysted bradyzoites, the extraintestinal (extraenteroepithelial) cycle begins with the development of tachyzoites in the lamina propria of the intestine of any mammalian host (3). The extraintestinal cycle constitutes the entire cycle in nonfelidae (intermediate) host. In cats it initially occurs simultaneously with enteroepithelial cycle (3, 4). Tachyzoites spread from cell to cell and are disseminated to various organs in macrophages, lymphocytes, granulocytes, and in free forms in the circulation. Most of these tachyzoites are arrested in lymph nodes, liver, and lungs, and a variable number enter the lymphatics and blood (3) (Table II). Encysted bradyzoites begin to appear in tissues in 1–2 weeks as immunity develops. In the cats this may coexist with the enteroepithelial cycle. Encysted bradyzoites develop intracellularly with particular affinity for neurons, retinal cells, cardiac and skeletal muscle.

Severity of the clinical syndrome produced is determined by the degree of cellular necrosis either directly due to the number of proliferating tachyzoites or indirectly because of hypersensitivity, or both. The clinical picture is varied, ranging from inapparent infections to lymphadenopathy or glandular toxoplas-

TABLE II
ROUTES OF DISSEMINATION



mosis, acute febrile toxoplasmosis, relapsing toxoplasmosis, encephalitis (particularly in immunosuppressed patients), toxoplasmosis during the third trimester of pregnancy with congenital infection, neonatal encephalitis, jaundice, or retinochoroiditis (22, 23).

Transmission

Although the definite mode of transmission is still not established, an extensive transmission scheme, based on the new life cycle of T. gondii has appeared in recent literature (3, 22, 23). The three known infective stages of Toxoplasma are:

1. bradyzoites
2. tachyzoites
3. sporozoites

The three modes of transmission are:

1. carnivorism
2. fecal contamination
3. transplacental or congenital infection

These modes of transmission involve the different infective stages as follows:

1. carnivorous ingestion of bradyzoites, tachyzoites of both (hamburger, uncooked mutton or beef)
2. contamination with feline feces containing sporozoites of sporulated oocysts.
3. transplacental infection of the fetus with tachyzoites, after ingestion of encysted bradyzoites or sporulated oocyst by the mother.

It should be noted that Toxoplasma oocysts, which are shed in feline feces, are unsporulated and are not infective until sporulated. Time needed for sporulation varies with temperature (24). Oocysts are resistant to acids, alkalies and common disinfectants but are killed by heat and 10% ammonia (24). Infective oocysts have been isolated from naturally contaminated soil. Land snails and earthworms (24), flies and cockroaches may serve as an intermediate host as a vector in transmission of the oocyst.

T. gondii is highly adapted to transmission by encysted bradyzoites via carnivorism. Bradyzoites are resistant to pepsin and trypsin digestion where tachyzoites may not survive gastric digestion (3).

Cats per se have been shown to be more readily infected with cysts of bradyzoites than oocysts (3, 8, 9, 24). Handling of raw meat infected with cysts of bradyzoites also has been implicated as a source of Toxoplasma infection (25).

Animals are known to transmit T. gondii transplacentally either in naturally occurring or experimentally induced infections. These include man, sheep, pigs, dogs, rats, mice, hamsters, guinea pigs, mink and cats (3, 5, 10, 13, 16–18, 20).

Generally, maternal infection and resulting immunity prior to pregnancy will protect subsequent fetuses. (However, Toxoplasma infections in two successive human pregnancies have been reported).

Toxoplasma also have been isolated from milk of cows, goats, pigs, dogs, rabbits, guinea pigs and mice with naturally occurring or experimentally induced infections (3).

Other potential means of transmission have been recorded as blood transfusion, organ transplants and laboratory and autopsy accidents (3, 16). Eggs of infected chickens rarely have been found to contain T. gondii (3, 16).

Epidemiologic Considerations

A. *Host Characteristics*

1. *Age:* The greatest concern, from the public health point of view, is neonatal toxoplasmosis in man. In the United States, an estimated 3,000 babies are born with the disease each year. It has been calculated that of these 3,000 babies, 5–15% die, 8–10% have marked brain and ocular lesions, 10–13% have moderate to marked visual damage. While 58–72% are clinically normal at birth, some of these develop active retinochoroiditis in childhood or young adulthood. It is estimated that the total annual cost of neonatal toxoplasmosis in the United States may be as much as thirty-one to forty million dollars (this includes, hospitalizations, institutionalization, and special education) (4). In general, surveys for Toxoplasma antibodies in animals and humans from many areas show that incidence of infection increases with age (26).

2. *Sex:* Numerous authors have tried to determine the prevalence rate based on sex differentation (25, 26). Some claim that there is a slightly higher prevalence rate for males. The sampling of random population in El Salvador revealed no significant difference in the prevalence rate of sexes so that even their statistical tests were run ignoring sex distribution (27).

Various population surveys in the United States have shown no difference in prevalence of positive titers in relation to sex (28). In some instances sex differences may be found due to bias in patient selection. In a statistical analysis of a series of selected patients in a New York Hospital who were observed by M. H. Edelman for possible clinical toxoplasmosis yielded the following sex differentiation (29).

Total Tested	102
Males	37
Females	65

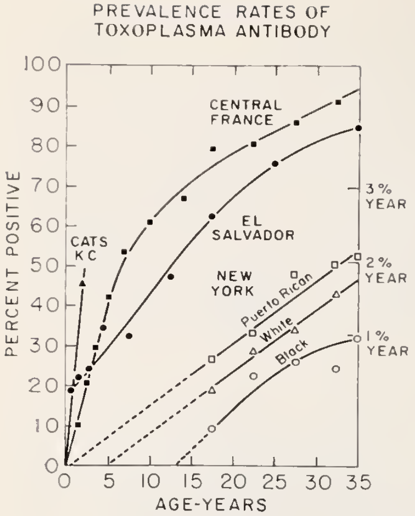


FIGURE 2

This is undoubtedly due to the concern over possible toxoplasmosis during pregnancy, and not due to any sex preference of the agent.

3. *Race and Ethnic Group Association:* In 1971 in New York, antibody prevalence rates of Black, Caucasian and Puerto Rican populations were tabulated by Kimball, Kean and Fuchs (9). They concluded that the prevalence rate is lower in the Black than Caucasian, and both are lower than the Puerto Rican population. This is most likely to be autochthonous to New York (Fig. 2). In other studies either the rate of infection is higher between 15 and 35 years than during childhood, or the manner of infection commences only in late childhood or adolescence (3).

It would be unwise to conclude any significant differences pertaining to race, since it is not known whether the population in question was a selected one or a random population used.

B. *Environmental Characteristics*

1. *Geographic areas:* It is a well-established fact that the rate of infection differs widely with localities. The incidence rate is highest in countries with hot, humid climates and the lowest incidence of Toxoplasma antibodies is found in inhabitants of dry, cold climates. It is interesting to note that no toxoplasma infection has ever been reported in Greenland or Alaska (30). This climatic association is understandable if one considers the fact that moisture favors transmission and dry and cold climate diminishes the viability of the Toxoplasma oocyst both in vivo and in vitro (5). The lesser incidence of Toxoplasma antibody at high altitudes may also be related to climate condition.

2. *Social and economic factors:* Consistency of the association of toxoplasmosis to higher socioeconomic groups is not even hypothesized in the literature at the present time. Culturally acquired carnivorism may be responsible for high prevalence rates in certain countries such as France and Haiti (3). While the

present studies are negative and inconclusive in this respect, it would be erroneous to assume that consumption of meat not thoroughly cooked is the main reason for the high prevalence. Ownership of pets, which might be higher in the upper socioeconomic group, can be considered to be only of subsidiary importance.

Until further information is collected about this disease entity and further in-depth epidemiologic studies are performed, no other environmental characteristics such as income, housing, occupation or education can serve one's hypothesis.

C. *Temporal Variation*

Temporal variation of toxoplasmosis at the present time can only be speculated upon. These speculations are usually based on reported cases on non-human Toxoplasma outbreaks either in seasonal or epidemic proportion.

Epidemic outbreaks of this disease in sheep in New Zealand or England, seasonal outbreaks which have occurred among ranch minks (18), are part of the present literature. Since one of the many intriguing aspects of toxoplasmosis is the similarity of expression between human and non-human infections, hypothetical speculations in this respect can be only temporarily assumed because the actual significance of these as they relate to the temporal variation of toxoplasmosis remains to be determined. I am convinced that this unclear point will keep many investigators or epidemiologists busy for years to come.

Serologic Tests

Serologic tests available for detecting toxoplasmosis include the Sabin-Felman dye test and the indirect fluorescent-antibody (ITA) test. These two tests detect early antibodies and usually yield positive results in 8 to 10 days after Toxoplasma infection. Other tests such as indirected hemagglutination (IgG) and complement fixation (CF) are also available.

In serologic testing of any species, emphasis is placed on demonstrating a rising titer at two-week intervals to confirm active Toxoplasma infection. In man, low antibody titers (1:4 for CF and 1:64 for IFA and dye test) generally indicate past infection. Intermediate titers (1:8 for CF and 1:256 for the other two tests) indicate recent infection, and high titers (1:32 for CF and 1:1,024 or 1:4,096 for others) suggest active or recent infection (3).

Prevention

Recommendations and guidelines for the prevention of toxoplasmosis in human beings, especially in pregnant women, can be summarized as follows:

1. Heat meat throughout to 66° C before eating.
2. Wash hands after handling raw meat.
3. If a cat is part of the household, feed indoor cats only dry, canned or boiled food and prevent them from hunting.
4. Flush cat feces down the toilet and scald litter pans daily or incinerate disposable litter trays daily.

5. Cover childrens' sandboxes when not in use and dispose of contaminated sand as required.
6. *Wear gloves* when handling litter pans and contaminated soil.
7. Avoid cats whose source of food is unknown (this applies especially to pregnant women).
8. Control cockroaches, flies, stray cats and rodents.

Periodic fecal examination and serologic surveillance of cats are not effective in detecting the infectious period of shedding oocyst. Control of diet is the only effective and practical method of preventing infection in cats.

Research on development of a vaccine for animals is encouraging, but extensive investigation of more than 50 isolants of Toxoplasma and other research are necessary to produce an effective vaccine (5).

Conclusion

Gaps in knowledge about occurrence and distribution of the disease are enormous. Just a few unanswered questions that are frequently asked follow. 1) How does man usually acquire infections? 2) How does he tolerate it so well? 3) What are the definite morbidity and mortality rates in human beings or animals? Congenital infections usually produce chorioretinitis, but to what extent does acquired infection produce similar disease? 4) How does the organism manage to persist in the host? 5) Does toxoplasmosis produce recurrent spontaneous abortions in women? 6) Should the finding of infection during pregnancy be viewed as an indication for therapeutic abortion? 7) Should a woman who has borne one child with congenital toxoplasmosis be considered a risk during subsequent pregnancies? 8) Why is the apparent appearance of dermal hypersensitivity delayed for so many months? 9) How can one account for the Toxoplasma infection in vegetarians? 10) Can infections be eradicated from man and other hosts?

Before definitive answers or explanations can be given to some of these questions, many years of intensive investigation will, no doubt, be required.

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Primary Melanoma of the Leptomeninges: A Review

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Introduction

Melanocytic elements of the pia mater may give rise to primary tumors of the central nervous system. The neoplastic process ranges from benign hyperpigmentation to malignant melanoma of the leptomeninges. Most reports have been single autopsy transcriptions, and little accord was reached by the various reviewers on the authenticity of individual cases. The principal areas of disagreement were criteria for diagnosis, terminology, classification of subdivisions, embryonal origin, and possibility of metastasis. The present review also takes under discussion physiological pigmentation, interrelation among the phacomatoses, and treatment.

Review of the World Literature

The first major review of the German literature was done by Omodei-Zorini (1) in 1924—a critical study of twenty patients, which yielded only twelve certain diagnoses. The primary compilation of case material by an American author was made in 1935 by Akelaitis (2), who evaluated seventeen additional cases and reckoned the proven total at thirty. Touraine's (3) analysis of fifty-nine primary pigmented tumors of the central nervous system appeared in the French literature in 1949; even with thirty-four more cases added, only forty-two were considered satisfactory clinical presentations. In 1957, Gibson, Burrows, and Weir (4) described another forty-five patients, accepting sixty-six autopsies overall as adequate. The next review of importance was by Kiel, Starr, and Hansen (5) in 1961; including forty-one new primary meningeal melanomas, no more than eighty-six were deemed valid. Major reports in the Japanese literature were published by Fukuda (6) in 1968 and by Maki, Minei, Nakada, Horie, and Ono (7) in 1969; thirty-five and twenty-seven cases were compiled respectively. A more thorough search of the world literature, including the Czechoslovakian, Finnish, French, German, Hungarian, Italian, Japanese, Polish, Russian, Spanish, Swedish, and Thai language journals, reveals over 300 primary melanomas of the leptomeninges, including eighty cases not previously mentioned in a review article.

Physiological Pigmentation

Melanin-containing cells are a constituent of many sites in addition to the corium of the skin: iris, ciliary body, choroid, and retina of the eye, chromaffin tissue of the carotid body and adrenal gland, cerebrospinal and sympathetic ganglia, labyrinth, reticular formation of the medulla, pons, subsantia nigra,

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locus ceruleus, and the leptomeninges. Dasgupta, Brasfield, and Paglia (8) also found primary melanomas in the following unusual loci: oral cavity, upper respiratory tract, esophagus, rectum, gallbladder, ovaries, cervix, vagina, and genitourinary tract. Meningeal melanocytes tend to be spindle-shaped with short arborizing processes and contain only a few granules of pigment. When numerous, the melanocytes together with phagocytes containing pigment migrate into the meningeal invaginations of the Virchow-Robin spaces and even into the actual brain substances (9). A few cases in the world literature may represent asymptomatic melanocytosis (10–17).

In 1836, Valentin (18) first described pigmented cells as elements of the leptomeninx in every elderly Caucasian and speculated on the physiological occurrence in the colored races of man. The ventral and lateral aspects of the medulla often have a silvery-grey appearance at autopsy, which may extend a variable distance over the spinal cord, brainstem, and base of the brain; Roca de Viñals, Elizalde-Armendáriz, and Coma-Fabrés (19) found such pigmentation in eighty-five of the one hundred cadavers in their study. Baader (20) noted that the characteristic distribution of malignant melanomas in the central nervous system follows the areas of normal pigmentation in the pia-arachnoid. In contrast to the human, pigment is most prominent in animals over the frontal lobes; this fact suggests that melanocytes accumulate where the cerebrospinal fluid tends to stagnate, as in dependent portions of the skull (21, 22). Meningeal pigmentation is increased during pregnancy in association with increased levels of melanocyte-stimulating hormone (23–26), but the statistical data does not support the theory that primary melanomas of the leptomeninges are more prevalent in women or during pregnancy or during the child-bearing years.

Heredofamilial Melanism

There are numerous examples in the white race of families with noticeably more pigmentation than average; dark tint of the iris and hair are associated with an overabundance of melanocytes in the lumbar area, buttocks, anogenital region, inguinal creases, thighs, axillae, scapular area, nipples, palpebrae, cheeks, and calves (3). Scheidt (27) made the first such observation in a family with fourteen cases in four generations. Two separate families exhibiting prominent skin nevi were studied by Orth (28); one with six cases in seventeen members in four generations, and the other with eight cases in fifteen members in four generations. Leber (29) gave the history of yet another family with hyperpigmentation in twenty-two cases in forty-five members in six generations. Central nervous system correlation was not documented until Van Bogaert (30) published the autopsy report of one of seven cases of cutaneous melanism in a family with thirteen other normally pigmented members; the patient was a forty-one-year-old man with a neuropsychiatric disorder, who expired after unsuccessful surgery to alleviate a strangled hernia. The genetic pattern in all five families followed regular Mendelian dominance.

Leptomeningeal Melanomatosis

In 1859, Virchow (31) reported the case of a thirty-year-old copperplater with recurrent painter's colic, who became partially paralyzed and blind; at necropsy, the diagnosis was made of melanotic sarcoma which had arisen in a primary diffuse melanosis and infiltrated almost the entire leptomeninx. Complete histologic sectioning in most published autopsies (Table I) revealed ex-

TABLE I

Case	Authors	Case	Authors
1	Virchow[a, b] 1859	33	Ehringer (35) 1958
2	Sternberg[b] 1903	34	Nikonova + Usova[e] 1958
3	Stoerck[a] 1904	35	Kanazawa[f] 1958
4	Thorel[a] 1907	36	Fasske[e] 1958
5	Boesch[a] 1912	37	Nakamura et al[g] 1959
6	Kiel[a] 1922	38	Uranova + Volodin[e] 1959
7	Hesse[a] 1923	39	Hokkaido[f] 1959
8	Matzdorff[b] 1923	40	Brodskii[e] 1959
9	Spencer[c] 1923	41	Tohoku[f] 1960
10	Neuberger[b] 1924	42	Cacudi + Cazzato (36) 1960
11	Omodei-Zorini (1)[h] 1924	43	Toranomon[f] 1961
12	Kraft[c] 1929	44	Piscol + Hoffman[f, h] 1961
13	Moren[d] 1930	45	Zangger (37) 1961
14	Lackerbauer[b] 1933	46	Spens et al (38) 1962
15	Garcin et al[b] 1933	47	Spens et al (38) 1962
16	Jacob[b] 1934	48	Olischer + Schroter (39)[h] 1963
17	Akelaitis (2) 1935	49	Likhashev (40) 1965
18	Becker[c] 1936	50	Tolnai[g] 1966
19	Winkelman et al[c, h] 1936	51	Tichy (14) 1966
20	Winkelman et al[c, h] 1936	52	Blumenbach + Trebbin (41) 1967
21	Salles[c] 1936	53	Cioffi et al (42) 1968
22	Benedeck (32) 1937	54	Satran + McDonald (43) 1968
23	Lence[d] 1937	55	Satran + McDonald (43) 1968
24	Gerstel (33) 1938	56	Satran + McDonald (43) 1968
25	Hule (34) 1946	57	Slaughter et al (44) 1969
26	Fatzer[d, h] 1951	58	Gluszcz + Lach (45) 1970
27	Russi et al[d] 1953	59	Gluszcz + Lach (45) 1970
28	Erbslöh[e] 1956	60	Andrioli (46) 1971
29	Erbslöh[e] 1956	61	Shuangshoti et al (47) 1971
30	Erbslöh[e] 1956	62	Bergdahl et al (48)[h] 1972
31	Erbslöh[e] 1956	63	Bergdahl et al (48)[h] 1972
32	Fischer[e] 1956	64	Savitz et al (49) 1974

[a] reviewed by Omodei-Zorini (1).

[b] reviewed by Akelaitis (2).

[c] reviewed by Touraine (3).

[d] reviewed by Gibson et al (4).

[e] reviewed by Kiel et al (5).

[f] reviewed by Fukuda (6).

[g] reviewed by Maki et al (7).

[h] criteria of Winkelman et al (113)

tensive infiltration of the subarachnoid space with perivascular cuffing and cranial nerve root involvement. Communicating hydrocephalus often resulted from blockage of the foramina of Lushka and Magendie in the cisterna magna as well as obliteration of the arachnoid villi over the convexity of the cerebral hemispheres. The age-distribution curve (Fig. 1) of these sixty-four cases plus an additional nine with metastases (Table V) demonstrates peak incidence in the fourth and fifth decades of life.

Neurocutaneous Melanosis

Rokitansky (50) described in 1861 a pigmented tumor of the entire brain and spinal cord in a fourteen-year-old girl, a hydrocephalic imbecile with a giant hairy nevus covering most of her trunk. The impression is widely held that ex-

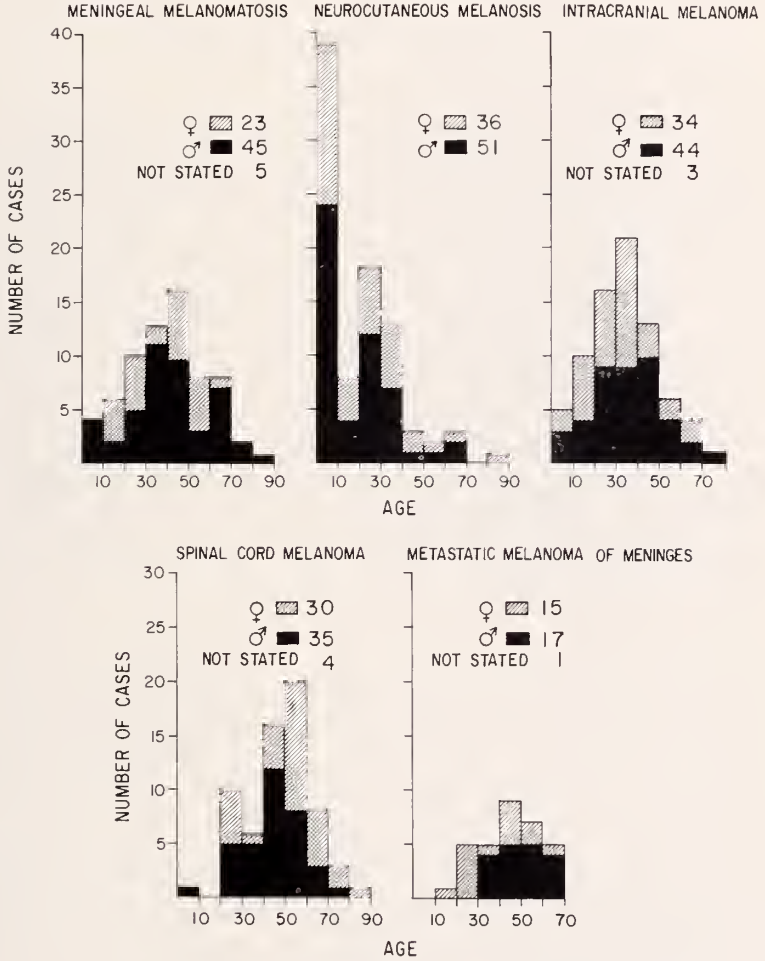

FIGURE 1

tensive pigmented nevi may predispose to malignant melanoma; Reed, Becker, Becker, and Nickel (51) reviewed 200 cases with giant nevi and found the reported incidence of malignancy to vary from 2 percent (52) to 13 percent (53); but in their own study of fifty-five cases, the incidence was as high as 31 percent. There was a marked tendency for extensive hairy nevi to follow a dermatome distribution from the posterior midline; designations such as "shoulder-stall," "cape-like," "coat-sleeve," and "stocking-like" were added to the original "bathing-trunk" and "vest-like." Upper cervical dermatome involvement was most frequently associated with hydrocephalus and leptomeningeal melanomatosis. Neurocutaneous melanosis (Table II) manifested simply large or numerous small skin nevi in more than half of these patients; of the sixty-seven cases plus five more with metastases (Table V) and fifteen others with solid neoplasms (Tables III and IV), about 45 percent occurred during the first decade, while 24 percent presented in neonates or in the first year of life (Fig. 1).

Intracranial Melanoma

Ogle (59) published in 1899 the autopsy of a thirty-two-year-old woman who followed a ten-week course of irritability, cephalgia, right hemiparesis, seizures, aphasia, emaciation, papilledema, and coma; the brain appeared as if painted over with sepia and lead, while a black globular tumor invaded the pineal gland, corpora quadrigemina, and thalamus. Whether or not anaplastic melanocytes from the meninges can penetrate neural tissue and form solid neoplasms remains a matter of controversy, but sixty-nine patients were reported with primary isolated intracranial melanomas (Table III) plus twelve others with metastases (Table V). The peak incidence was in the third and fourth decades (Fig. 1). The clinical presentation varied accordingly with the neuroanatomical location. In addition to the listed tumors, pigmented glioma (69), pigmented meningioma (25, 70–74), pigmented medulloblastoma (75), pigmented acoustic neuroma (76), primary melanoma of the hypophysis (77, 78), and primary melanoma of the choroid plexus (79) have all been reported; but the authors offered alternate theories of histogenesis.

Spinal Cord Melanoma

Hirschberg (80) and Pick (81) in 1906 both investigated the same spinal cord tumor in a sixty-seven-year-old woman who became paraplegic; an intramedullary melanophoroma was discovered at the T12-L1 level with extensive involvement of the pia mater. Sixty-one primary melanomas of the spinal cord (Table IV) were reviewed plus eight additional cases with metastases (Table V). The presenting symptomatology was predominantly that of thoracic cord compression. There were thirty-six intramedullary, thirty-one intradural, and two extradural tumors. Half of these cases occurred in the fifth and sixth decades (Fig. 1). Pigmented meningiomas originating in the spinal area have also been described (25, 72, 94, 95, 96).

TABLE II

Case	Authors	Case	Authors
1	Rokitansky[a] 1861	35	Juntendo[f] 1962
2	Jablokoff + Klein[g] 1879	36	Okinaka + Noda[f] 1962
3	Frank[g] 1903	37	Alajouanine et al[f] 1963
4	Grahl[a] 1906	38	Ketels-Harken[f] 1963
5	Oberndorfer[a] 1908	39	Nishiyama + Shinoda[f] 1963
6	Schlesinger + Schopper[a] 1912	40	Musger[f] 1963
7	Binsack[c] 1912	41	Lund[f] 1963
8	McLachlan[b] 1913	42	Ariwa + Fukuda[f] 1964
9	Lua[a] 1914	43	Keio[f] 1964
10	Berblinger[a, h] 1915	44	Keio[f] 1964
11	Heilmann[b] 1931	45	Fox et al[f] 1964
12	Farnell + Globus[b] 1931	46	Fox et al[f] 1964
13	Björnboe[c] 1935	47	Fox et al[f] 1964
14	Netherton[c] 1936, Bakody et al[e] 1950	48	Willis (151)[h] 1965
15	Weidman + Eiman[c] 1936	49	Tveten[f] 1965
16	Kessler[c] 1937	50	Tveten[f] 1965
17	Jutte[d] 1939	51	Reed et al[f] 1965
18	Lecouterier et al[c] 1939	52	Hjelt + Velska (54) 1966
19	Wilcox[c] 1939	53	Nagao et al[g] 1966
20	Larue et al[g] 1944	54	Hoffman + Freeman[g] 1967
21	Russell[d,h] 1949	55	Hoffman + Freeman[g] 1967
22	Vivano[d] 1953	56	Hasegawa et al[g] 1968
23	Vivano[d] 1953	57	Terao et al (55) 1968
24	Ishizu + Kato[f] 1955	58	Terao et al (55) 1968
25	Fanconi[f] 1956	59	Williams (56) 1969
26	Fanconi[f] 1956	60	Dugois et al (57) 1969
27	Abe et al[g] 1957	61	Slaughter et al (44) 1969
28	Bouton[e, h] 1958	62	Slaughter et al (44) 1969
29	Osima et al[g] 1959	63	Slaughter et al (44) 1969
30	Nakamura et al[g] 1959	64	Maki et al (7) 1969
31	Brodskii[e] 1959	65	Deshpande et al (58)[h] 1970
32	Kawasaki[f] 1961	66	Deshpande et al (58)[h] 1970
33	Schultz[f] 1961	67	Bergdahl et al (48)[h] 1972
34	Tokyo[f] 1962		

[a] reviewed by Omodei-Zorini (1).
[b] reviewed by Akelaitis (2).
[c] reviewed by Touraine (3).
[d] reviewed by Gibson et al (4).
[e] reviewed by Kiel et al (5).
[f] reviewed by Fukuda (6).
[g] reviewed by Maki et al (7).
[h] criteria of Winkelman et al (113).

Metastatic Melanoma of the Meninges

In 1905, Pol (97) examined the case of a fifty-nine-year-old man who presented with right hemiparesis, cephalgia, prominent nevi, and cervical adenopathy; post mortem findings included meningeal hyperpigmentation, three separate melanomas in one frontal lobe, and multiple metastases to the esophagus,

TABLE III

Case	Authors	Case	Authors
1	Ogle[d] 1899	36	Gibson et al (4) 1957
2	Minelli[a, h] 1906	37	Kubota et al[g] 1957
3	Hamill + Rothstein[a] 1907	38	Jikei[f] 1958
4	Weimann[c, h] 1923	39	Nakamura et al[g] 1959
5	Dieckmann[b] 1929	40	Brodskii[e] 1959
6	Foot + Zeek[b] 1931	41	Uranova + Volodin[e] 1959
7	Bailey + Bucy[c] 1933	42	Haraszti + Gomba (12) 1960
8	Rohde[d] 1935	43	Chiba[f] 1960
9	Shapiro + Kellert[c] 1937	44	Krihara et al[g] 1961
10	Jutte[d] 1939	45	Noda et al[g] 1962
11	Moersch et al[d] 1940	46	Matsumoto et al[g] 1963
12	Moersch et al[d, h] 1940	47	Mark (60) 1963
13	Moersch et al[d] 1940	48	Ariwa + Fukuda[f] 1963
14	Saito et al[g] 1940	49	Balasubramaniam + Ramamurthi (61) 1964
15	Christensen[d] 1941		
16	Christensen[d] 1941	50	Iwate[f] 1964
17	Mackay + Hurteau[d, h] 1942	51	Heda et al[g] 1965
18	Cardona[d] 1947	52	Dailly[f] 1965
19	Bailey[e] 1948	53	Nagao et al[g] 1966
20	Willis[d] 1948	54	Kilwashita[g] 1967
21	Björkesten[d] 1949	55	Morris + Danta (62)[h] 1968
22	Woringer + Gloor[d] 1950	56	Lewis (63) 1968
23	Herzog[d] 1950	57	Sugita[g] 1968
24	Albertengo[d] 1951	58	Hasegawa et al[g] 1968
25	Thurel et al[e] 1952	59	Terao et al (55) 1968
26	Barontini[d] 1953	60	Terao et al (55) 1968
27	Kawase et al[g] 1953	61	Terao et al (55) 1968
28	Halpert[d] 1953	62	Medoc + Folle (64)[h] 1969
29	Bachtiarow[d] 1953	63	Lewis (65) 1969
30	Roca de Viñals et al[e] 1954	64	Kinoshita et al (66) 1970
31	Roca de Viñals et al[e] 1954	65	Mathew et al (67) 1970
32	Sawaki et al[g] 1955	66	Bergdahl et al (48)[h] 1972
33	Erbslöh[e] 1956	67	Bergdahl et al (48)[h] 1972
34	Tohoku[f] 1956	68	Bergdahl et al (48)[h] 1972
35	Gros + Roilgen[e] 1956	69	Limas + Fernin (68) 1972

[a] reviewed by Omodei-Zorini (1).
[b] reviewed by Akelaitis (2).
[c] reviewed by Touraine (3).
[d] reviewed by Gibson et al (4).
[e] reviewed by Kiel et al (5).
[f] reviewed by Fukuda (6).
[g] reviewed by Maki et al (7).
[h] criteria of Winkelman et al (113).

lymph nodes, mediastinum, mesentery, stomach, intestine, liver, and testicles. Leptomeningeal melanomatosis may metastasize outside the central nervous system, but a cutaneous growth which has undergone spontaneous regression is more likely (9). Dasgupta et al (8) found metastases to the brain in 39 percent of patients dying of malignant melanoma, while Pack (98) found cerebral

TABLE IV

Case	Authors	Case	Authors
1	Hirschberg[a, h] 1906, Pick[a, h] 1906	32	King + Propst[d, h] 1951
2	Esser[a, h] 1907	33	Fatzer[d] 1951
3	Kawashima[c, h] 1910	34	Declich[e] 1952
4	Lindbom[a, h] 1912	35	King et al[d, h] 1952
5	Koelichen[b] 1916	36	Perino[d] 1953
6	Ford + Feror[c] 1924	37	Roca de Viñals et al[e] 1954
7	Ringertz[c] 1926	38	Erbslöh[e] 1956
8	Ringertz[c] 1926	39	Gros + Roïlgen[e] 1956
9	Schmid[b, h] 1926	40	Leger et al[e] 1957
10	Bau-Prusak + Mackiewicz[d] 1929	41	Gibson et al (4)[h] 1958
11	Bell[c] 1930	42	Zimmerman + Adams[e] 1959
12	De Blasi[b, h] 1930	43	Lang + Bridge[e] 1959
13	Van Bogaert + Verbrugge[c] 1933	44	Uranova + Volodin[e] 1959
14	Foerster + Gagel (82) 1935	45	Hirano + Carton (86)[h] 1960
15	Neri + Serra[c] 1936	46	Piscol + Hoffman[f, h] 1961
16	Von Törne (83) 1938	47	Kiel et al (5) 1961
17	Da Costa + Love[d] 1939	48	Pappenheim + Bhattacharji (87) 1962
18	Moersch et al[d] 1940		
19	Moersch et al[d] 1940	49	Chugunov et al (88) 1965
20	Moersch et al[d] 1940	50	Raukhman (89) 1966
21	Rasmussen et al[e] 1940	51	Raukhman (89) 1966
22	Rasmussen et al[e] 1940	52	Lin + Cook (90) 1966
23	Rasmussen et al[e] 1940	53	Tolnai et al[g, h] 1966
24	Garcin[c] 1941	54	Beresford (91) 1968
25	Woods + Pimenta[e] 1944	55	Mathew et al (67) 1970
26	Kissel et al[d] 1950	56	Becker et al (92) 1970
27	Castaner et al[d] 1950	57	Vuori + Hormia (93) 1972
28	Forbes + Maloney[d] 1950	58	Vuori + Hormia (93) 1972
29	Sicard + Juliard (84) 1950	59	Vuori + Hormia (93) 1972
30	Woltman et al (85) 1951	60	Bergdahl et al (48)[h] 1972
31	Lamartine de Assis + De Luccia[e] 1951	61	Bergdahl et al (48)[h] 1972

[a] reviewed by Omodei-Zorini (1).
[b] reviewed by Akelaitis (2).
[c] reviewed by Touraine (3).
[d] reviewed by Gibson et al (4).
[e] reviewed by Kiel et al (5).
[f] reviewed by Fukuda (6).
[g] reviewed by Maki et al (7).
[h] criteria of Winkelman et al (113).

involvement in 38 percent of the autopsies on patients with metastatic melanoma. The presence of visceral pigmented lesions should indicate an overwhelming probability that the primary site was not in the meninges. Of the thirty-three cases collected (Table V), the distribution was fairly regular from the third through the seventh decades (Fig. 1). Malignant melanomas of dermal origin occur most frequently between thirty and sixty years of age, and the incidence is rare before puberty (107). In contrast, 45 percent of the meningeal melanomas without metastases became symptomatic in the first three decades.

TABLE V

Case	Authors	Case	Authors
1	Pol[a] 1905	18	Oliveras de la Riva + Norin-Mora (101) 1936
2	Boit[a] 1907		
3	Ehnmark + Jacobowsky[c] 1926	19	Pautler + Gallavan[e] 1951
4	Hassin + Bassoe[e] 1928	20	Russi et al[d] 1953
5	Baumeker[b] 1929	21	Zwozdiak[e] 1955
6	Vigano[c] 1930	22	Busch (102) 1958
7	Vigano[c] 1930	23	Nikonova + Usova[e] 1958
8	Foot + Zeek[b] 1931	24	Nikonova + Usova[e] 1958
9	Gordon[c] 1932	25	Tohoku[f] 1959
10	Görög (99) 1932	26	Brodskii[e] 1959
11	Burns-Plewes[c] 1935	27	Brodskii[e] 1959
12	Burns-Plewes[c] 1935	28	Salm (103) 1967
13	Schnitker + Ayer[c] 1938	29	Clifford et al (104) 1968
14	Conzett + Langford[e] 1940	30	Holaday et al (105) 1968
15	Mackay + Hurteau[d] 1942	31	Voog et al (106) 1968
16	Wilkins + Halpert (100) 1946	32	Bergdahl et al (48) 1972
17	Strong[e] 1947	33	Bergdahl et al (48) 1972

[a] reviewed by Omodei-Zorini (1).
[b] reviewed by Akelaitis (2).
[c] reviewed by Touraine (3).
[d] reviewed by Gibson et al (4).
[e] reviewed by Kiel et al (5).
[f] reviewed by Fukuda (6).

Venous invasion was an unusual feature of such neoplasms (108), but some evidence of limited invasive ability has been presented: along the needle track of a ventricular puncture (37), and via a lumboperitoneal shunt (4).

Criteria for Diagnosis

In 1897, Ribbert (109) maintained that all pigmented tumors of the brain, spinal cord, and meninges are secondary. Reports of widespread central nervous system involvement by Pilliet (110) in 1887 in the case of a previously excised malignant melanoma of the skin and by Dobbertin (111) in 1900 in a patient with prior enucleation of a tumor-ridden eye led Lubarsch (112) in 1920 to believe that no melanotic neoplasm could be considered primary if there was even one cutaneous nevus. In 1936, Winkelman, Gotten, and Silverstein (113) made allowance for inconsequential pigmented moles and established the most widely accepted principles: the presence of a diffuse melanoblastic infiltration of the soft membranes of the brain, and adequate necropsy including the orbital contents to verify the absence of any other source.

Solitary metastases from dermal melanomas are common, and often the original site cannot be ascertained. The patient may deny ever having had a lesion removed, and careful search of the skin and squamous mucosa and eyes may prove fruitless. Before making the diagnosis of primary melanoma of the leptomeninges, there should be clear histologic evidence that melanocytosis preceded melanomatous transformation (9).

Documentation could be established for only forty-one patients, whose autopsy included sectioning of the eyes; the vast majority of postmortem examinations were incomplete. The diagnosis was made solely by surgical biopsy in forty-three cases, by clinical course in four cases, and by cerebrospinal fluid cytology in one case (114). The thirty-three metastatic melanomas of the meninges must by their very nature be considered separately.

Embryonal Origin

The early literature abounds with controversy over the histogenesis of melanocytes confined to the central nervous system. Virchow (31) in 1859 considered pigment cells in the meninges derivatives of the mesoderm and postulated that these connective tissue corpuscles might proliferate and gradually assume the characteristics of a diffuse melanosarcomatosis. In 1877, Obersteiner (115) established the occurrence of melanophores in the adventitia of pial vessels which penetrate into the medullary substance. Unna (116) believed in 1893 that only epithelial cells produce pigment and irregularly split off from the basal layers as congenital crests which may later form carcinomas. In 1907, Wieting and Hamdi (117) differentiated between the melanophoromas which arose from mesenchymal phagocytes and the melanoblastomas which owed their origin to pigment-forming cells of the ectoderm. Spencer (118) maintained in 1923 that scattered foci of pigmented cells found occasionally in deeper tissues developed around the neural tube originally for the protection of the central nervous system. In 1926, Masson (119) theorized the derivation of melanoblasts from sensory nerve endings. Ewing (120) supported this theory in 1930 and advanced the idea of superficial melanomas arising from the cells of Merkel-Ranvier bodies. In 1931, Foot and Zeek (121) demonstrated the presence of meningeal nerve fibrils with end-organs similar to Wagner-Meissner corpuscles in two melanotic neoplasms of the leptomeninges.

In 1910, Harrison (122) studied the growth of amphibian nerve fibers in tissue culture and noted that pigmented cells frequently formed from pieces of medullary cord. Dorris (123) found in 1939 that transplanted neural crest from embryos produced donor coloration in host feathers. In the fishes and reptiles, some evidence for the origin of melanophores from the anlage of the central nervous system was presented by Lopashov (124) in 1944. In 1948, Rawles (125) studied the migration of mammalian neural crest tissue to the epidermis, iris and choroid of the eye, and the leptomeninges; she conclusively demonstrated that, when neural crest was excluded from transplants, skin and hair were structurally normal but free of pigment.

Terminology

The nomenclature applied to pigmented lesions of the meninges by various authors has been more than ample:

1. melanosis: Virchow (31), 1859
2. melanism: Touraine (3), 1949
3. melanocytosis: Erbslöh (114), 1956
4. melanosarcomatosis: Stoerk (126), 1904

5. melanocytoblastosis: Dieckmann (127), 1929
6. melanoblastosis: Farnell and Globus (128), 1931
7. melanocarcinomatosis: Foot and Zeek (121), 1931
8. melanomatosis: Burns-Plewes (129), 1936
9. melanoblastomatosis: Cardona (130), 1947
10. melanocytomatosis: Erbslöh (114), 1956
11. melanoma: Pilliet (110), 1887
12. melanocarcinoma: Unna (116), 1893
13. melanophoroma: Ribbert (109), 1897
14. melanosarcoma: Ogle (59), 1899
15. melanoperithelioma: Minelli (131), 1906
16. melanoblastoma: Wieting and Hamdi (117), 1907
17. melanoepithelioma: Spencer (118), 1923
18. melanocytoblastoma: Omodei-Zorini (1), 1924
19. melanocytoma: Hassin and Bassoe (132), 1928

Prominent but asymptomatic pigmentation of the central nervous system was described by Touraine (3) as 'melanism,' and by Erbslöh (114) as 'melanocytosis.' Virchow intended 'melanosis' to convey a marked hyperplasia of the pigmented mesodermal elements of the leptomeninx. However, the term, 'melanomatosis,' introduced by Burns-Plewes (129), has come into general use for abnormal pigmentation of the meninges with pathologic sequelae. The various theories of histogenesis were each represented by a correlated name for the neoplasm; but the original, 'melanoma,' has come to be accepted as most appropriate.

Interrelation among the Neurocutaneous Syndromes

The term 'hamartoma' was coined by Albrecht (133) in 1904 as a definition of tumor-like masses resulting from faulty embryonal development of cells and tissues natural to the organs in which the nodules occur. In 1923, Van der Hoeve (134) introduced the word 'phacomatosis' to define diseases hereditary and developmental in origin which produce a host of symptoms characterized by the presence of spots, cysts, and tumefactions plus other congenital malformations in various parts of the body, and especially in the nervous system. Thus, tuberous sclerosis, von Hippel-Lindau disease, Sturge-Weber-Dimitri syndrome, von Recklinghausen's neurofibromatosis, and neurocutaneous melanosis were grouped together as neuroectodermal dysplasias (135, 136).

Histological evidence of cutaneous neurofibromatosis was presented in three patients (44, 137, 138), and multiple café-au-lait spots were seen in four others with primary melanoma of the leptomeninges (49, 57, 58, 114). Examples of neurocutaneous hemangiomatosis with leptomeningeal melanosis were given in five necropsy reports (10, 45, 139, 140, 141). These simultaneous occurrences of two forms of neuroectodermal dysplasia would seem to demonstrate a close association among the phacomatoses—a theory advanced by Soldan (142) in 1899. On the other hand, single coincidences of multiple sclerosis (143), acoustic neuroma (63), pontine glioma (137), rhabdomyosarcoma (44), pituitary adenoma (101), and even three coincidental syringomyelias (144, 145, 146) are not out of the realm of statistical probability.

Prognosis and Treatment

Kiel et al (5) calculated that with involvement of the brain the median course of symptoms was five months and the median postoperative survival one month; with involvement of the spinal cord, the median course of symptoms was six months and the median postoperative survival four months. One patient was asymptomatic for ten months following excision of an intradural cervical melanoma and survived another ten months after implantation of a ventriculoperitoneal shunt to relieve internal hydrocephalus. Longer postoperative courses up to ten years were observed in the cases of Moersch, Love, and Kernohan (147), Leger, Pouyanne, Got, and Guerin (148), Vuori and Hamdi (93), and Lin and Cook (90). Radiation therapy alone has been essentially ineffective in almost all patients, but Vuori and Hamdi (93) also reported survival for more than four years after incomplete removal of an intramedullary thoracic melanoma and treatment with 4,680 rad.

Three- to eight-month amelioration with steroid therapy in patients with malignant melanoma metastatic to the central nervous system has been attributed by Beresford (91) to the reduction of cerebral edema. Chugunov, Utkin, and Donskoi (88) recorded generalized improvement in neurologic status continuing at twelve months following laminectomy and chemotherapy with cyclophosphamide for an intradural thoracic melanoma. In addition, clinical remissions of neurologic symptoms have been produced in malignant melanoma with the use of a new cytotoxic agent, imidazole carboxamide (149). For the future, further investigation of the immunogenic properties of such neoplasms may prove rewarding (150–154).

Summary

In addition to the 100 cases of primary melanoma of the leptomeninges reviewed in the American and European journals (48), over 200 reports have been compiled from the entire world literature for a better understanding of this rare neoplasm. The different manifestations of the neurocutaneous syndrome are presented with statistical data. The criteria for diagnosis, histogenesis, nomenclature, prognosis, and interrelation with the other phacomatoses are discussed.

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Malignant Changes in Seborrheic Keratoses

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Malignant transformation of a seborrheic keratosis, contrary to the opinion of some authorities (1, 2), does take place (3, 4). During the course of the past five years I have observed a total of 19 such instances taking the form either of basal cell or in situ carcinomas.

Results of Case Study

The cases are summarized in Table I. All lesions occurred in Caucasians with no history of arsenic intake and had been present for many years. There appeared to be no significant sex predilection (11 males and 8 females) and the average age was 66 years (involving patients from the fourth to the ninth decades). All cases except one (number 8) showed signs of recent inflammation.

Sixty-eight percent of the cases occurred on the head and 32% involved the temple. Twenty-six percent of the malignancies were basal cell carcinomas while 74% were in situ carcinomas. All of the basal cell carcinomas except one arose from seborrheic keratoses showing a predominant acanthotic pattern (Figs. 1 and 2). The one exception developed from a seborrheic keratosis of the hyperkeratotic type (Figs. 3 and 4). All of the in situ carcinomas except four occurred within seborrheic keratoses of the hyperkeratotic type (Figs. 5 and 6). Two of the four exceptions developed within the reticulated type, and the remaining two arose within the acanthotic type. Basaloid cell melanin was a prominent feature in 63% of the cases and solar elastosis was conspicuous in 45%.

Comment

Malignant changes do occur in seborrheic keratoses and are not rare. In all instances of basal cell carcinomas arising from seborrheic keratoses, no evidence of basal cell carcinoma was identified histologically within the adjacent normal skin. The development of basal cell carcinomas, although well defined and characteristic histologically, seem to be less likely than in situ carcinomas. The latter range from small, inconspicuous microscopic foci (Figs. 5 and 6) to striking extensive histologic features indistinguishable from Bowen's disease (Figs. 7 and 8). The presence of these malignant changes merging with microscopic fields of uniform basaloid cells in all of these cases represents strong evidence that a number of similar cases may have been misdiagnosed as Bowen's disease in the past.

Since malignant changes do not seem to be rare, why then have there been so few cases documented in the medical literature? How does one know that

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TABLE I

*Malignant Changes in Seborrheic Keratoses**

Case	Sex	Age	Location	Description of Lesion	Clinical Impression	Microscopic Diagnosis
1	M	67	Scalp	Irregular, brown, crusted firm nodule (1.5 cm)	SK or BCC	BCC arising from SK, AT
2	F	83	Outer canthus	Circular, tan ulcerated nodule (0.8 cm)	SK or BCC	BCC arising from SK, AT
3	M	64	Temple	Irregular, brown, verrucous tender plaque (2.0 cm)	SK or BCC	SK, RT, with in situ CA
4	F	55	Abdomen	Irregular, tan, verrucous crusted plaque (1.5 cm)	SK	SK, HT, with in situ CA
5	M	40	Temple	Oval, tan, verrucous ulcerated plaque (1.0 cm)	IN	SK, HT, with in situ CA
6	M	60	Temple	Irregular, tan, verrucous tender plaque (1.6 cm)	SK or BCC	SK, HT, with in situ CA
7	M	59	External ear	Circular, skin-colored, verrucous crusted plaque (1.0 cm)	BCC	BCC arising from SK, HT
8	F	67	Cheek	Irregular, brown verrucous plaque (1.6 cm)	SK	SK, HT, with in situ CA
9	M	76	Leg	Irregular, tan, verrucous tender plaque (2.0 cm)	SK	SK, HT, with in situ CA
10	F	66	Leg	Oval, skin-colored, verrucous tender plaque (1.5 cm)	SCC	SK, HT, with in situ CA
11	M	67	Forehead	Circular, brown, firm tender nodule (1.2 cm)	SK	SK, AT, with in situ CA
12	M	79	Forehead	Irregular, brown, crusted firm nodule (2.2 cm)	BCC	SK, RT, with in situ CA
13	M	65	Temple	Oval, brown, verrucous tender plaque (2.0 cm)	SK or BCC	SK, HT, with in situ CA
14	F	73	Cheek	Irregular, skin-colored, verrucous crusted plaque (1.2 cm)	SCC	SK, HT, with in situ CA
15	F	57	Forearm	Circular, skin-colored, verrucous tender plaque (1.6 cm)	SCC	SK, HT, with in situ CA
16	M	83	Temple	Oval, brown, ulcerated firm nodule (1.2 cm)	BCC	BCC arising from SK, AT
17	F	52	Shoulder	Irregular, brown, crusted firm nodule (1.5 cm)	SK or BCC	BCC arising from SK, AT
18	F	70	Temple	Irregular, brown, verrucous tender plaque (1.6 cm)	SK	SK, AT, with in situ CA
19	M	77	Buttock	Circular, tan, verrucous tender plaque (1.4 cm)	SK	SK, HT, with in situ CA

* SK, seborrheic keratosis; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; IN, intradermal nevus; CA, carcinoma; AT, acanthotic type; HT, hyperkeratotic type; RT, reticulated type; M, male; F, female; measurement in cm indicates maximum dimension or diameter.

what I have diagnosed as in situ carcinomas represent true malignancies in the absence of dermal invasion? There are a number of possible valid answers to the first question. Many seborrheic keratoses are removed without a biopsy being performed. With poorly preserved and improperly prepared sections, early in

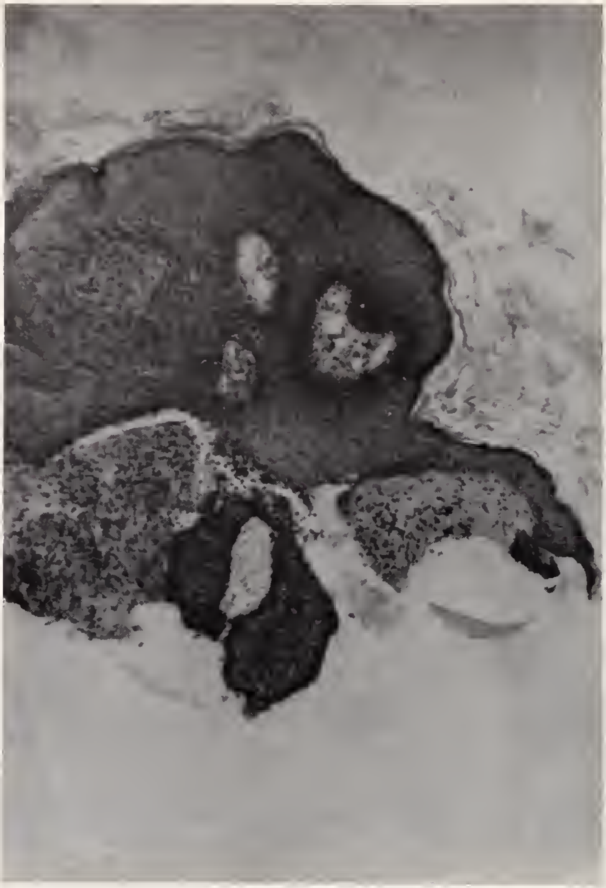

FIG. 1. Case 2 showing basal cell carcinoma arising from base of acanthotic seborrheic keratosis (H&E × 40).

situ carcinomas which require careful scrutiny with the best of preparations will surely be overlooked. Finally, not only do I believe, as mentioned above, that a number of cases have been misdiagnosed as Bowen's disease in the past, but also I feel certain that there are cases on record that have been diagnosed as invasive cutaneous squamous cell carcinomas which originated in seborrheic keratoses, particularly some of the verrucous forms of carcinoma. In these instances, the entire seborrheic keratosis has probably been transformed into in situ carcinoma. Also, for these very same reasons, it is not possible to determine a true incidence of such malignant changes. To reply to the second question, one simply has to turn to the identical histologic criteria required for diagnosing in situ carcinomas involving other body tissues, a most notable example being that of carcinoma in situ of the cervix uteri. Since these are accepted as malignancies

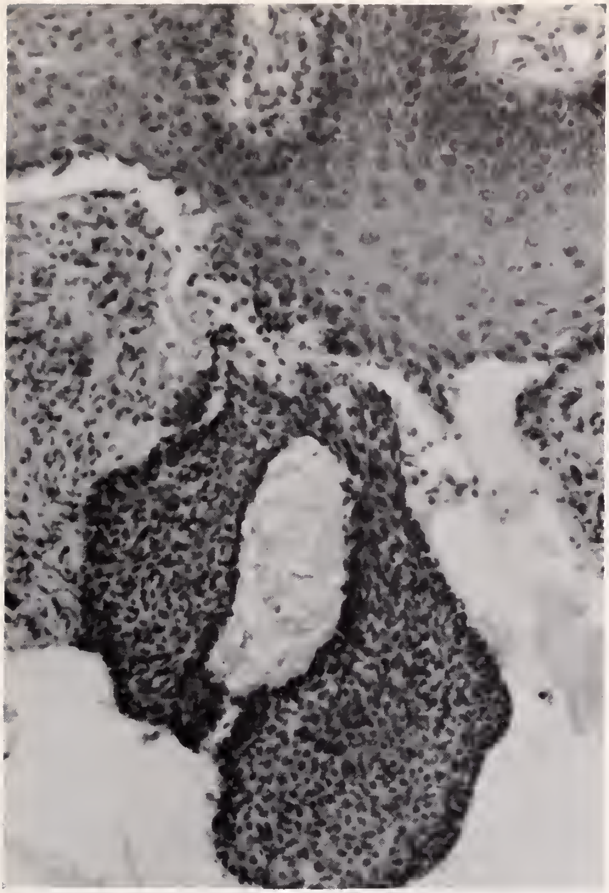

Fig. 2. Higher magnification of Fig. 1 (H&E × 100).

by all qualified pathologists, then why make an exception with a cutaneous lesion? In addition, Bowen's disease, from which the histologic features may be indistinguishable, is accepted as an in situ carcinoma which presents a characteristic clinical picture and which is considered to be caused by a systemic carcinogen. Needless to say, for the benefit of the patient, it is important to diagnose cancer as early as possible so that thorough removal can be performed with a minimal degree of scarring and a better prognosis.

It is significant that approximately one-third of the cases (32%) involved the temple. In about two-thirds of the cases (63%) a seborrheic keratosis was considered in the clinical diagnosis. It seems reasonable to conclude from these findings that a skin lesion which appears to represent a seborrheic keratosis showing clinical signs of recent change should be biopsied so that removal can be performed should malignant changes prove to be present.

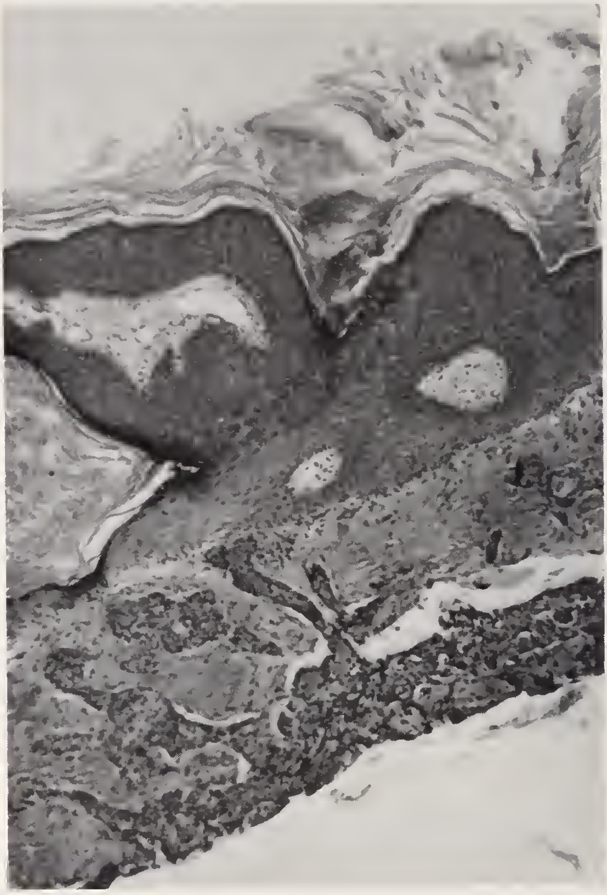

FIG. 3. Case 7 showing basal cell carcinoma arising from base of hyperkeratotic seborrheic keratosis (H&E × 40).

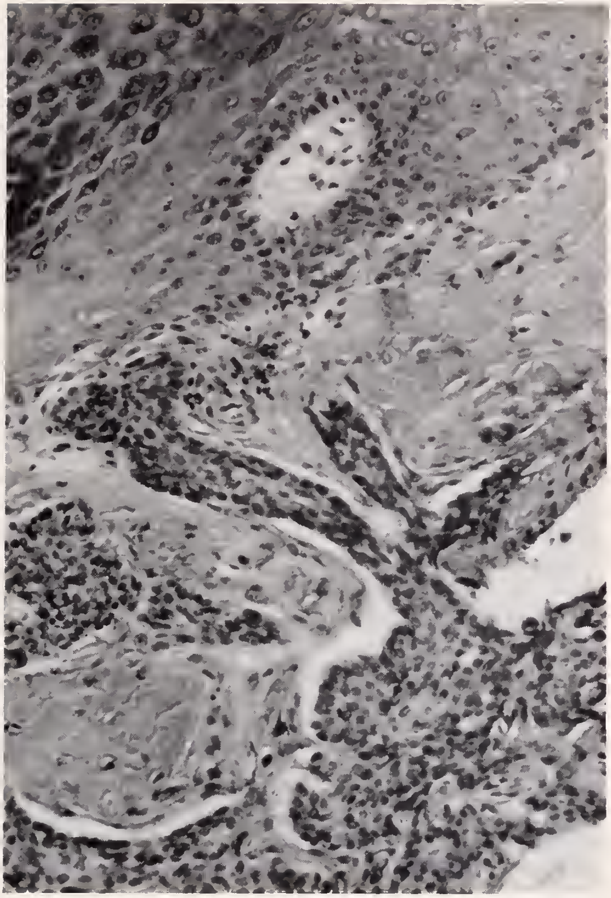

Fig. 4. Higher magnification of Fig. 3 (H&E × 100).

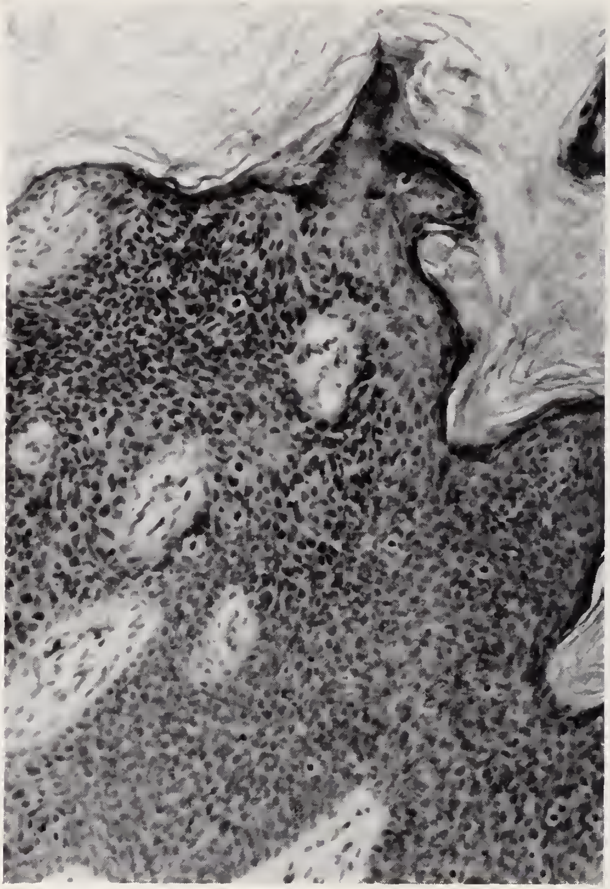

FIG. 5. Case 11 showing relatively inconspicuous but well-defined foci of in situ carcinoma arising from acanthotic seborrheic keratosis (H&E ×100).

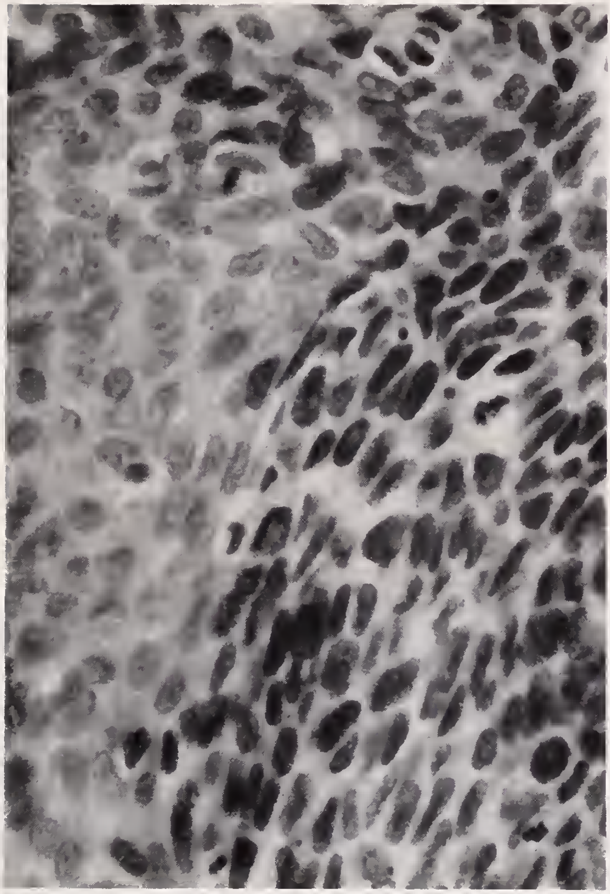

FIG. 6. Higher magnification of Fig. 5 (H&E × 400).

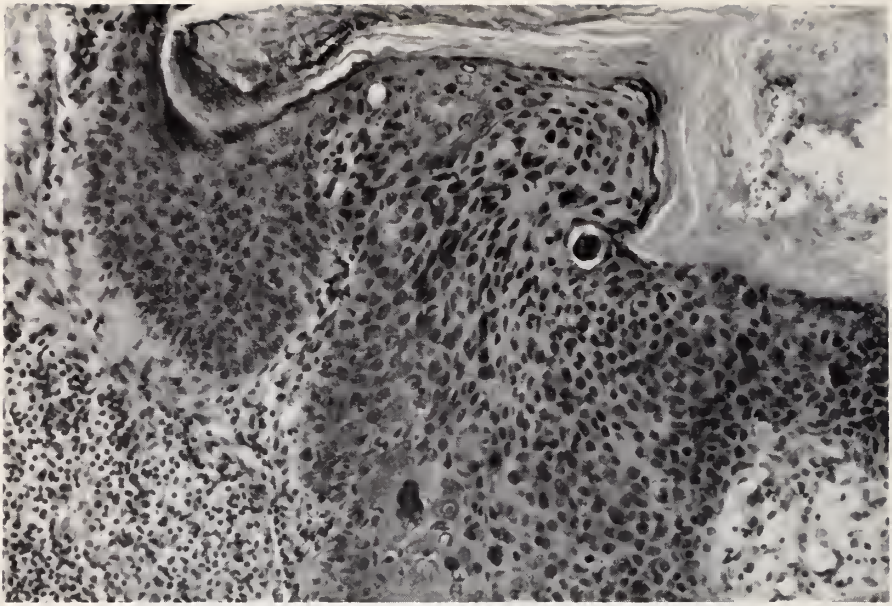

FIG. 7. Case 13 showing in situ carcinoma simulating Bowen's disease arising from hyperkeratotic seborrheic keratosis (H & E × 100).

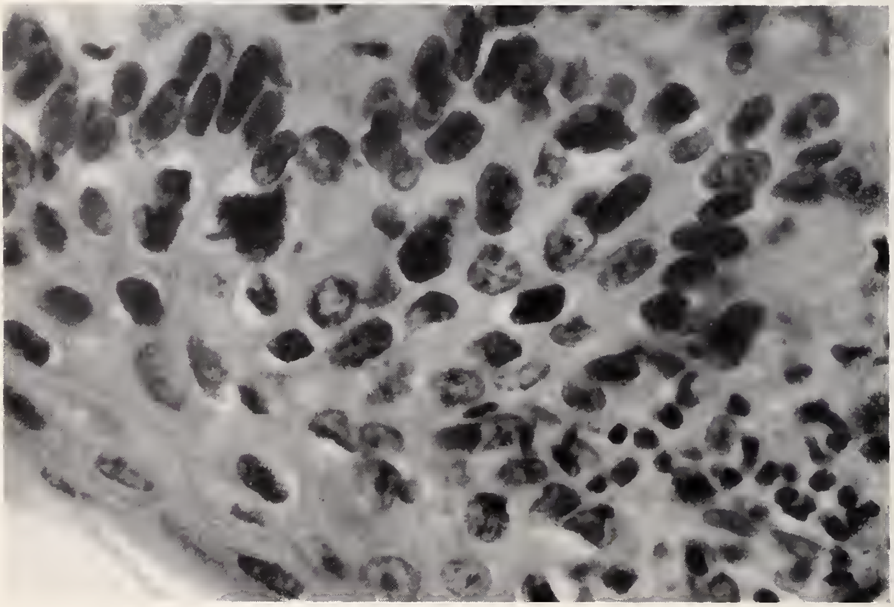

FIG. 8. Higher magnification of Fig. 7 (H&E × 400).

Summary

Malignant transformation of 19 seborrheic keratoses has been summarized and described, the malignant change representing either basal cell carcinoma or an in situ carcinoma. Some cases similar to the latter may have been misdiagnosed in the past as Bowen's disease. Some verrucous squamous cell carcinomas probably represent total malignant transformation of seborrheic keratoses with invasion. Since such changes do occur and do not seem to be as uncommon as one might expect, it is imperative that all changing seborrheic keratoses be biopsied so that thorough removal can be performed should malignant changes prove to be present.

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Diffuse Lymphosarcoma of the Colon Simulating Ulcerative Colitis

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Extensive malignant lymphoma predominantly involving the colon is rare. Rosenberg et al (1) found that in 1,269 cases of malignant lymphoma of the GI tract, there was involvement of some part of the colon in only 84 cases. Of these, diffuse involvement was present in only a small minority. The rarity of this type of lymphomatous involvement of the colon has been observed by others (2, 3, 4). In more than one-half of these cases, the disease also involved the small intestine or the small intestine and the stomach. Diffuse lymphosarcoma of the colon may simulate chronic ulcerative colitis (1, 5–9). We are reporting such a case in order to review features which distinguish it from chronic ulcerative colitis.

Case Report

M.G., a fifty-eight-year-old white woman was in good health until June of 1971 when she experienced the onset of bloody diarrhea. She was then admitted to a hospital where a barium enema was performed, and reported to be normal. On sigmoidoscopy, however, thickened mucosal folds with ulcerations were seen in the distal portion of the sigmoid. Biopsy of the mucosa was reported as "lymphoid hyperplasia." Nonetheless, the patient was thought to have ulcerative colitis, and she was treated with corticosteroid retention enemas. The patient experienced transient symptomatic relief with this therapy, but thereafter she began to experience progressively more frequent intermittent bloody diarrhea. During the months prior to her admission to The Mount Sinai Hospital in January 1973, she experienced four to six watery and sometimes bloody stools each day, and had marked malaise, easy fatigability, and a ten-pound weight loss. In addition, she had noted a lump in the right inguinal region of several months' duration. Her past medical history was of interest in that she had undergone a partial thyroidectomy thirty years earlier for reasons that were unknown to her.

Physical examination revealed a chronically ill-appearing, slightly pale, middle-aged white woman. Her temperature was 99°F, and other vital signs were normal. A firm mass was palpated in the right inguinal region 5 cm in diameter and there was slight right lower quadrant tenderness.

Pertinent laboratory studies: hemoglobin 11 gm, WBC 8,900 mm^3 with a differential count of 45% polymorphonuclear leucocytes, 52% lymphocytes and 3% monocytes, platelets 387,000 mm^3. Urine analysis was normal, as were the blood electrolytes and BUN. Serum albumin was 3.1% gm. Alkaline phosphatase was 100 IU. Examination of the stool revealed no ova or parasites. Stool culture revealed only normal fecal flora. Chest x-ray was normal.

Sigmoidoscopy revealed the mucosa diffusely studded with 5 mm diameter round, slightly raised nodular lesions which on biopsy were found to be infiltrated with small round cells. This was considered to be diagnostic of small cell lymphosarcoma. Barium enema initially was interpreted as "diffuse ulcerative colitis." Multiple fine serrations at the margins of the barium column were seen. However, subsequent review of the film yielded the opinion that these findings were more compatible with diagnosis of diffuse lymphosarcoma of the colon.

Colonoscopy with the Olympus CFMB revealed the same mucosal lesions as had been

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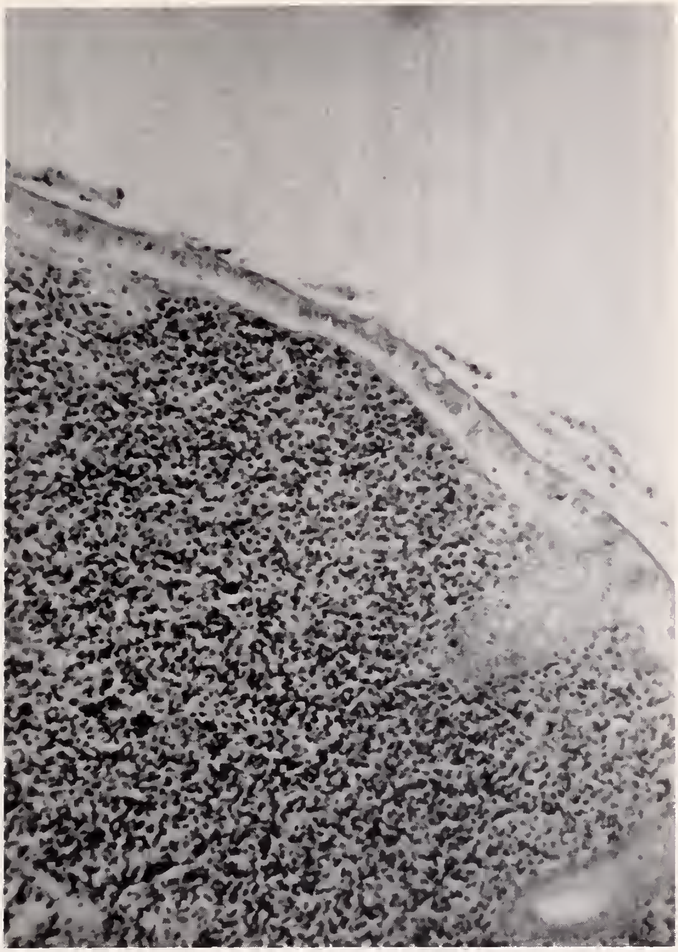

Fig. 1. Colonic mucosa showing infiltration by small cell lymphosarcoma.

seen by sigmoidoscopy, and they extended throughout the entire colon. The regularity of the nodulation produced a grid-like or waffle-iron type of appearance. Biopsies were taken in the sigmoid, transverse and distal ascending colon, and each proved to show lymphosarcoma.

Lymphangiogram demonstrated large nodes in the iliac area compatible with involvement by malignant lymphoma. Upper GI and small intestinal x-ray study demonstrated irregularity due to small nodular masses in the terminal ilium. Bone marrow examination was normal. On review of the original rectal biopsy slides, they were felt to be compatible with the diagnosis of lymphosarcoma.

The patient improved symptomatically on antidiarrheal treatment and was subsequently started on chemotherapy with vincristine, prednisone and cytoxan. There was a weight gain and a general feeling of well-being with a subsequent decrease in diarrhea. The mass in the right inguinal region disappeared with therapy.

Discussion

Classification of malignant lymphomas generally recognizes three categories which are determined by the predominant cell type (10, 11): lymphosarcoma,

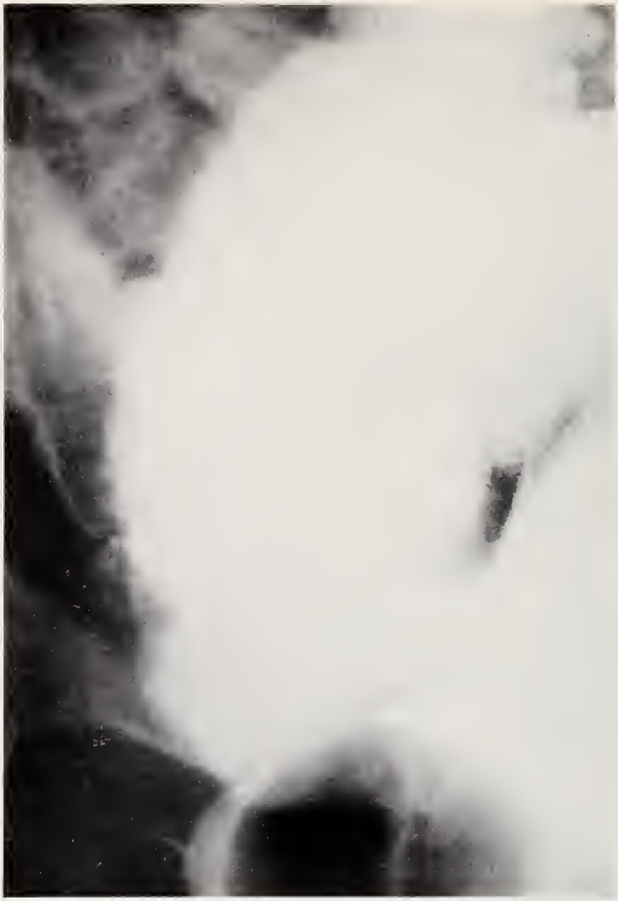

FIG. 2. Barium enema. Note serrations of the margins of the barium column, absence of shortening of the colon, and the large caliber of the lumen.

reticulum cell sarcoma and Hodgkins disease. Lymphoma primary in the colon represents only 0.5% of all malignant tumors of this organ (8). Secondary involvement of the colon by lymphoma is substantially more common (1). Lymphomas involving the colon manifest themselves in one of three patterns: segmental mucosal lesions, diffuse polypoid lesions and bulky extra colonic (usually pelvic) masses. The diffuse polypoid form, which is the variety that mimics ulcerative colitis, is almost always due to lymphosarcoma (8). Lymphosarcoma is the least common of the malignant lymphoma to involve the colon (12). The diffuse submucosal infiltrating form of lymphosarcoma is less common than that presenting as a discrete or localized mass (2).

A number of factors add to the difficulty in differentiating diffuse lymphosarcoma of the colon from chronic ulcerative colitis: 1) Lymphoid hyperplasia or diffuse lymphoid infiltration is often seen in mucosal biopsies in ulcerative colitis and can be confused with the mucosal histologic appearance in lympho-

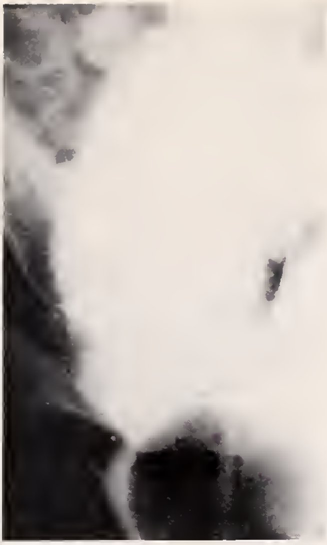

FIG. 3. Magnification of sigmoid colon barium enema.

sarcoma of the colon, as occurred initially in our patient; 2) Diffuse submucosal infiltration by lymphosarcoma leads to the development of diffuse polypoid lesions which on sigmoidoscopy and barium x-ray can be mistaken for pseudopolyps of ulcerative colitis. Superficial ulcerations may on occasion also be present; 3) lymphosarcoma of the colon and ulcerative colitis can occur together in the same patient (8, 13).

The radiographic similarities and differentiating features of ulcerative colitis and lymphosarcoma of the colon have been well reviewed in the literature (3, 9, 14) and can be briefly summarized. Diffuse lymphosarcoma of the colon may show a polypoid or cobblestone appearance on barium enema examination. The margins of the barium column may be serrated with the mucosa also having a finely reticulated appearance. These changes may be similar to some of those seen in chronic ulcerative colitis. However, in lymphosarcoma, there is no shortening of the colon or loss of haustral pattern, nor irritability of the colon during filling with barium, and no significant mucosal alterations are present. In lymphosarcoma of the colon there may be short, flat, scalloped areas and the lumen is often increased in size with failure of collapse and apposition of the colon walls following evacuation of barium. Finally, in lymphosarcoma, there are associated lesions in the small intestine and the stomach.

The colonoscopic observations in our case were very helpful in delineating the extent of the colonic lesions and clearly demonstrated the absence of ulceration or other features of chronic ulcerative colitis. We have been unable to find any other reports in the literature of colonoscopic findings in diffuse lymphosarcoma of the colon*. This endoscopic procedure with biopsy appeared to us to be a useful adjunct to sigmoidoscopy in differentiating lymphosarcoma from chronic ulcerative colitis.

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* A more detailed discussion of our colonoscopic finding as well as color pictures is in press.

Because of the extent of the involvement of the disease surgery was not feasible in our patient. Although radiotherapy is a recognized and useful treatment for lymphosarcoma, the extent of the disease process led us to utilize systemic chemotherapy initially (2, 3, 8).

Summary

A case is reported of lymphosarcoma which extensively and predominantly involved the colon and simulated ulcerative colitis for almost two years before the correct diagnosis was made. This type of colonic lymphosarcoma is rare. The literature dealing with this subject is reviewed, including the diagnostic features differentiating it from ulcerative colitis.

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Treatment of Rheumatoid Arthritis with Gold

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Abstract

Fifty-four patients with rheumatoid arthritis were treated with intramuscular injections of gold salts. Thirty-two achieved significant improvement. There were 12 instances of toxicity. The method of treatment and the possible adverse effects are discussed.

Over the past quarter century, investigations into the nature of rheumatoid arthritis have yielded much information. Yet the treatment still leaves much to be desired. Corticosteroids, anti-malarials and various anti-inflammatory and analgesic agents such as phenylbutazone and indomethacin have made contributions, but their values are limited by insufficient benefit or the drug cannot be continued because of major toxic or undesirable physiologic reactions. Treatment of rheumatoid arthritis by chrysotherapy became popular in Europe following Forestier's pioneer publication in 1929 (1). In the United States, it fell into disrepute because of numerous reports of serious toxicity. In retrospect, much of this may have resulted from excessive dosage, indiscriminate use, and insufficient awareness of the dangers. There was a renewed interest in gold salt therapy, in great part due to Freyberg's investigations (2, 3), following World War II. It is now accepted by most authorities as a major modality in treatment of rhematoid arthritis. An important contribution was the controlled study of chrysotherapy by the British Empire Rheumatism Council, which clearly indicated definite value (4).

However, many physicians still do not generally accept the use of gold salts because of the highly exaggerated fears of adverse reactions. Too few clinical reports are published. This article outlines experiences treating with gold salt injections 54 patients suffering from rheumatoid arthritis. Comparatively few developed toxicity, and a majority achieved significant and satisfactory improvement.

Choice of Subject

Age and sex were of no consequence; in this series all were adults except one teenager with psoriatic arthropathy. All patients should receive full benefits of available physiotherapy and rehabilitation, and regulation of rest and activity. As a general rule, active cases of rheumatoid arthritis should be given an adequate trial with salicylates. When this has failed, chrysotherapy can be initiated. It must be clearly understood that only early-stage, active rheumatoid arthritis (the first 2 years are optimum) stands to benefit from chrysotherapy. In the "burned out," the chronic arthritic patient with gross deformities and ankylosis, gold salts are valueless. In juvenile rheumatoid arthritis, however, it may be helpful. When the diagnosis of rheumatoid arthritis is in doubt, i.e.,

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in mild and atypical forms of "arthritis," gold salts should not be used. Although psoriatic arthropathy is not likely to be benefited, and, indeed, there is some evidence that the psoriasis may be aggravated, a cautious trial of chrysotherapy is not contraindicated. This is particularly true when the psoriasis and rheumatoid arthritis are separate entities in the same patient. A past or present history of hepatic or renal disorders, or a blood dyscrasia, interdicts the use of gold. Serious allergies, particularly those to metals, should mandate extra caution in the prescription and administration of gold salts.

Method of Treatment

A number of gold salt compounds are available, none of them of greater benefit or producing fewer ill effects than the others. Colloidal gold preparations are non-toxic, but have absolutely no therapeutic value. The two most commonly used preparations are: Myochrisine Injection® (gold sodium thio-malate, 50% gold) Aqueous solution and Solganol (Gold thio-glucose, 50% gold) Suspension. They are equally effective and with similar toxic reactions. Solganol is slightly less painful on injection, but is more difficult to draw into the syringe. Occasionally, a patient will develop an immediate postinjection vasomotor reaction to one salt, mainly tachycardia and flushing. This quickly disappears; recurrent reaction can be prevented by switching to the other product.

Before each injection of gold salt, the physician should inquire regarding gastrointestinal symptoms, pruritus, dermatitis, mouth and throat soreness, purpura and gingival bleeding. A urinalysis and complete blood count are done. If no adverse reactions are detected, the injection can be given.

The initial dose is 10 mg followed by 15 mg the next week, then 25 mg weekly for 4 injections, and 50 mg each week thereafter. Improvement is not likely before 300 to 400 mg total has been administered. Indeed, early rapid improvement, e.g., by 75 mg, may be a warning of impending toxicity and an indication for extremely close observation. Weekly injections are continued to a total of 1000 mg. If at this level no significant improvement is noted, gold therapy should be discontinued. However, if sufficient improvement is seen, treatment can be continued every 2 weeks for a month, and then every 3 or 4 weeks indefinitely. When the clinical status is stable, injections may alternate between 25 and 50 mg or be kept at 35 mg. Any sign of increased rheumatoid activity indicates a return to 50 mg doses.

If treatment is stopped after a remission has occurred, the improvement may persist for varying periods. If and when the arthritis returns, a second course of gold is often not as effective. In only one instance has it been necessary to discontinue long-term therapy. This patient, after receiving 2150 mg gold salts, developed hepatitis. This case is reported as gold toxicity, but infectious hepatitis cannot be excluded. As seen in Table I, many patients have received chrysotherapy for years without ill effects.

Gold Toxicity

A great advantage of chrysotherapy is that peptic ulceration is not produced or activated, as is often the case with indomethacin and phenylbutazone, nor is

TABLE I

Patients on Long-Term Gold

More than 10,000 mg	1
More than 7,000 mg	2
More than 5,000 mg	2
More than 2,500 mg	7

seen the host of Cushingoid changes associated with corticosteroids or the serious eye problems induced by anti-malarials. The frequent, serious ill effects of immunosuppressive drugs restrict their use to the desperately ill rheumatoid patient, in whom all else has failed. (5) Yet, gold salt therapy does present problems:

1. The most common is dermatitis. This may vary from a faint eruption on the extremities to a severe exfoliation. The latter is rare and usually can be avoided by proper attention to early symptoms, discontinuation of gold therapy, and observation of the course of the rash. Any dermal reaction preceded by or accompanied by pruritus should be considered related to the gold.
2. The most serious toxic reaction is bone marrow depression with thrombocytopenia, granulocytopenia, anemia and aplastic anemia. The sudden appearance of a sore throat or fever may antedate a leukopenia and should suggest stopping gold treatment. A white blood cell count dropping below 4000 WBC/cmm. demands cessation of gold injections and observation with daily blood counts. Rapidly worsening leukopenia indicates the need for hospitalization, antibiotics, cortisone, and possibly transfusions. These methods usually suffice, but if not, BAL (British AntiLewisite) should be given.
3. Renal toxicity may present as albuminuria and/or red blood cells in the urine.
4. Hepatitis and enteritis occur, but rarely.
5. Stomatitis and a metallic taste are seen independent of blood dyscrasia. Following a serious blood dyscrasia, persistent urinary abnormalities, hepatitis, or severe dermatitis, chrysotherapy should never be resumed. After transient albuminuria or a mild dermatitis, gold treatment can be reintroduced cautiously; daily dosage, however, should not exceed 25 mg. In all patients in whom toxicity developed, the symptoms and signs appeared prior to the administration of a total of 400 mg gold. The only exception was the patient with hepatitis mentioned above.

Results

Fifty-four patients have been treated by intramuscular injections of gold salts. Twelve did not derive benefit and treatment was terminated at 1000 mg. Toxicity developed in 12 instances (Table II) and injections were discontinued. Two of these patients with dermatitis maintained the improvement indefinitely. In 3 patients, several months after the dermatitis healed, the arthritis having recurred, chrysotherapy was reinstituted with remission. Including these 5 patients, 32 patients in all achieved a satisfactory response (6) (Table III).

TABLE II

Gold Toxicity

Dermatitis	9
Leukopenia	1
Microscopic hematuria	1
Gold (?) hepatitis	1

TABLE III

Results

Grade I	Complete remission	17
Grade II	Major improvement	13
Grade III	Minor improvement	2
Grade IV	Unimproved or worse	12

TABLE IV

Class IV to I	1 patient
Class III to I	14 patients
Class II to I	5 patients
Class III to II	12 patients
Unchanged or worse	12 patients

One patient after a 6-year remission to Grade I, gradually relapsed and despite all treatment terminated in Grade IV. A second patient remained in Grade I for 2 years and then also relapsed into Grade IV. Their sedimentation rates were very rapid and the latex agglutination titres quite high. These 2 cases are included in the 12 "unimproved"; but considering the length of remission in each, they are also among the 32 who improved. Using the Steinbrocker Functional Class (6), the results can be presented differently. Classes I through IV outline the patient's ability to perform his usual and necessary functions for living. Any shift from one class to the next would indicate a significant therapeutic gain or loss. Table IV presents the results in this series.

All patients who developed toxicity recovered completely. The white blood cell count of the patient who developed leukopenia had dropped rapidly to 900/cm. but with no other adverse signs. She was hospitalized, treated prophylactically with penicillin and made a rapid and uneventful recovery. As already noted, there were 12 cases who developed toxicity and in whom treatment was stopped.

Summary

Since there is no known curative treatment for rheumatoid arthritis, a medication which offers reasonable opportunity for palliation without excessive risk, should be tried. An experience with observed improvement of 32 out of 54 patients given a regimen of parenteral gold supports its usefulness in treating rheumatoid arthritis.

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NEUROLOGIC NOTES

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Primary Leptomeningeal Melanomatosis Presenting as Carcinomatous Meningitis

Submitted by

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Introduction

Among the infrequent tumors encountered in neuropathology, one of the rarest is primary melanoma of the leptomeninges. Only forty case reports appearing in the world literature were well documented by extensive autopsy including the eyes; and the exact nature of the neoplasm was not established in the majority of patients until after death (1–15). In the present case, while the patient was still living the diagnosis was made on the basis of cerebrospinal fluid cytology, neuroradiology studies, and brain biopsy and was confirmed by complete postmortem examination.

Case Report

A twenty-seven year old nurse presented with staggering gait, left temporal cephalgia, and emesis. For four weeks prior to admission, she experienced episodic vertigo, nausea, and a low-pitched noise in the left ear. Significant in her past medical history was psychiatric treatment for depression over a two-year period.

Physical examination revealed a pale, lethargic, dehydrated young woman with multiple café-au-lait spots over her trunk and extremities. Neurologic testing demonstrated a mild organic brain syndrome and symmetrical paraparesis. Left finger-to-nose dystaxia and bilateral incoordination on heel-to-shin maneuvering were noted. There was clockwise rotary nystagmus and bilateral papilledema with hemorrhages and exudates.

Initial complete blood count, urinalysis, serology, serum chemistries, LE phenomenon, antinuclear antibody, and sedimentation rate were within normal limits or negative, as were x-rays of the chest and skull and an electrocardiogram. Skin tests for tuberculosis, histoplasmosis, and coccidiomycosis were nonreactive. Multiple Gram stains, India ink preparations, and cultures of blood, sputum, urine, and cerebrospinal fluid failed to show any organisms. Febrile aggultinins and viral and fungal serum antibody titers were also negative.

Twenty-two lumbar punctures were performed during her hospital stay. The pressure was never below 300 mm of water, the sugar content was persistently low with a minimum of 20 mg% and a maximum of 42 mg%, the protein content rose continuously over a six week period to a high of 140 mg%, and the cell count ranged from lymphocytes 2/mm^3 to 24/mm^3. Cerebrospinal fluid cytology revealed numerous clusters of pigmented malignant cells, which were concentrated by membrane filter technique (Fig. 1).

Electroencephalography was diffusely abnormal, left more than right side. Brain scan was interpreted as normal. A pneumoencephalogram showed minimal dilation of the basal cisterns, cisterna ambiens, and pericallosal space with paucity of air over the convexities.

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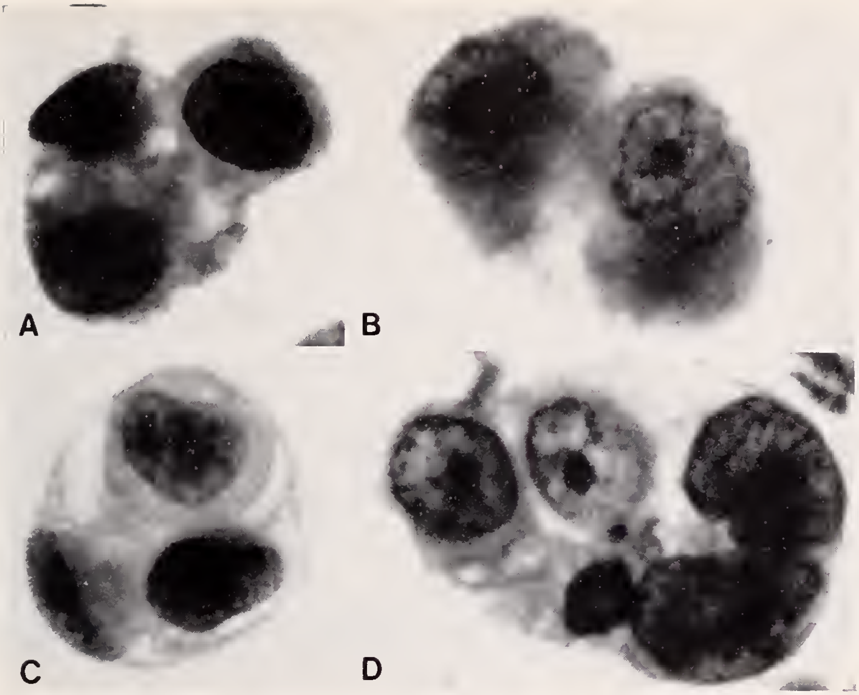

Fig. 1. Four clusters of malignant cells filtered from the cerebrospinal fluid, exhibiting large nuclei, prominent nucleoli, crowded chromatin structure, and coarse cytoplasmic pigment granules (Papanicolaou × 2430).

Bilateral brachial angiography visualized numerous engorged and tortuous veins on the anterior aspect of the brainstem and cerebellum, but no mass lesion was defined (Figs. 2 and 3).

Dexamethasone therapy for cerebral edema continued throughout her hospitalization. Multiple cranial nerve deficits developed, beginning with left VI, VII, VIII and right VI and progressing to left III, IV, V, VI, VII, VIII, IX, X and right VI, VII, VIII, X. Following several generalized seizures, diphenylhydantoin therapy was begun. She became increasingly disoriented and experienced distress from inability to swallow her secretions. Tracheostomy was performed, and intravenous alimentation instituted.

On the thirty-sixth hospital day, a right temporal craniotomy was performed for a brain biopsy. The meninges were diffusely infiltrated by well-differentiated tumor cells containing black-brown granular pigment which gave positive reactions with the ferrous ion technique. Postoperatively, she remained dyspneic and spiked a fever of 40.6° C; serial chest x-rays demonstrated bilateral bronchopneumonia and pulmonary edema. She continued to follow a febrile course; on the forty-third hospital day, she experienced cardiorespiratory arrest and expired.

Complete postmortem examination was performed, and no extraneural metastases were found. The leptomeninges contained focal and confluent areas of grey-brown discoloration, which were particularly concentrated over the ventral surfaces of the brainstem, in the interpeduncular cistern, cisterna ambiens, and pontine and medullary cisterns in the region of the lateral recesses (Fig. 4). Transverse sections through the cerebrum, cerebellum, and brainstem disclosed severe generalized hydrocephalus and well-circumscribed zones of similar discoloration in the underlying cortex (Fig. 5).

Histological examination of representative paraffin sections from all cerebral lobes, thal-

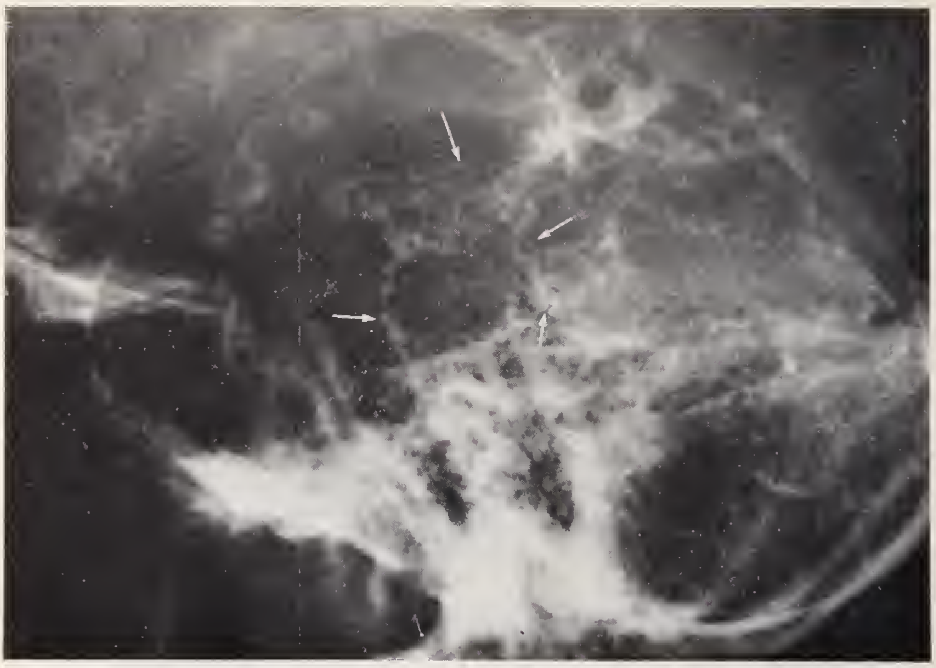

Fig. 2. Bilateral brachial angiogram, lateral view. Irregularities in the posterior mesencephalic, anterior pontomesencephalic, lateral mesencephalic, precentral, and supraculminate veins are demonstrated (arrows).

amus, and hypothalamus, corpus striatum, midbrain, pons, cerebellar vermis and hemispheres, medulla, and spinal cord showed diffuse infiltration of the leptomeninges by melanin-containing cells similar to those observed in the biopsy; in the more concentrated areas, small meningeal veins were often focally compressed (Fig. 6). Immediately subjacent zones of cerebral cortex were frequently invaded along the course of penetrating parenchymal vessels (Fig. 7). Aggregates of tumor cells were also encountered in various subependymal zones, notably the periaqueductal grey matter where local infiltration caused compression of the cerebral aqueduct. Cell form and differentiation varied from area to area of the tumor: the cells over the cerebral convexities resembled hyperplastic melanocytes, while the cells over the ventral surfaces of the cerebellum and brainstem displayed a neuron-like pattern more typical of malignant melanoma (Fig. 8).

Distal segments of most cranial nerves were infiltrated by tumor cells, and neurons of peripheral ganglia often showed chromatolysis (Fig. 9). Neuromelanin in neurons of the substantia nigra, locus ceruleus, tegmentum of the medulla, and peripheral ganglia was normally distributed. The anterior lobe of the pituitary gland was also invaded as were the leptomeninges covering both optic nerves. Numerous sections of the retinae, the skin, and the gastrointestinal tract failed to reveal any other primary or secondary neoplasm.

Discussion

Diffuse involvement of the leptomeninges, presenting as cephalgia, dementia, and multiple cranial nerve palsies and evidenced by increased pressure, lymphocytosis, elevated protein, and decreased sugar content of the cerebrospinal fluid, may be seen in the following conditions:

1. carcinomatosis
2. leukemic infiltration

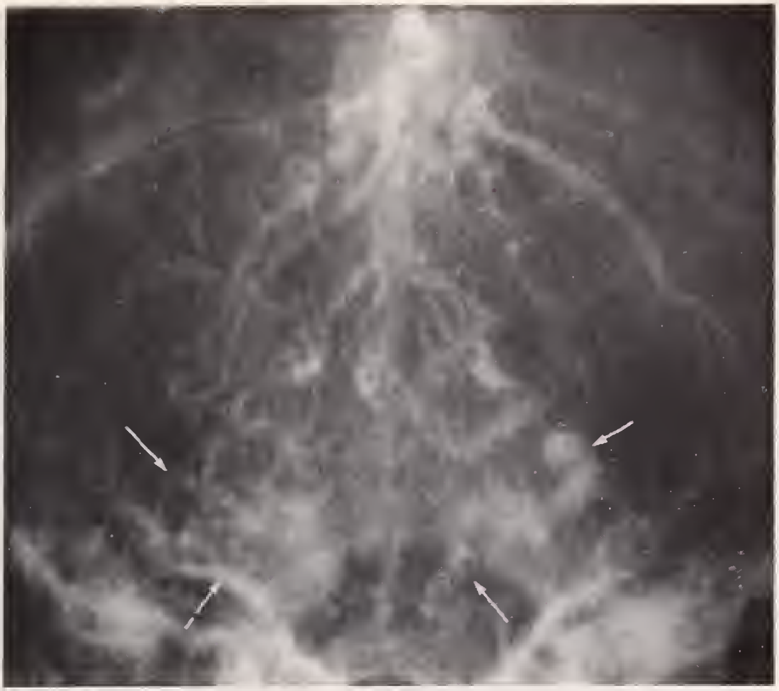

FIG. 3. Bilateral brachial angiogram, Towne view. Numerous inflamed veins are visualized in the basal cisterns, particularly in the cerebellopontine angles (arrows).

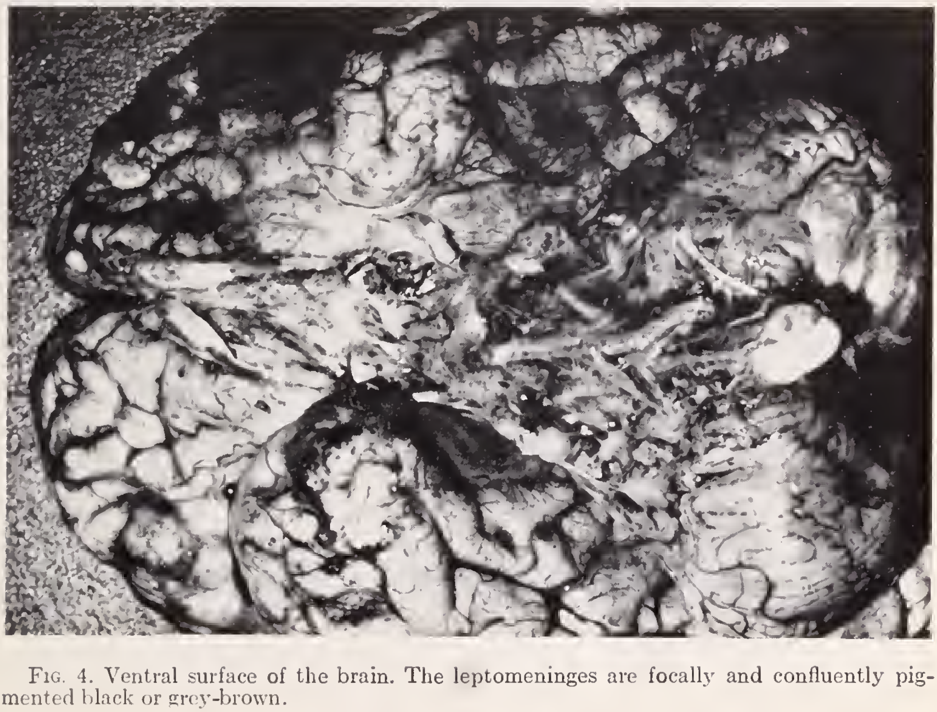

FIG. 4. Ventral surface of the brain. The leptomeninges are focally and confluently pigmented black or grey-brown.

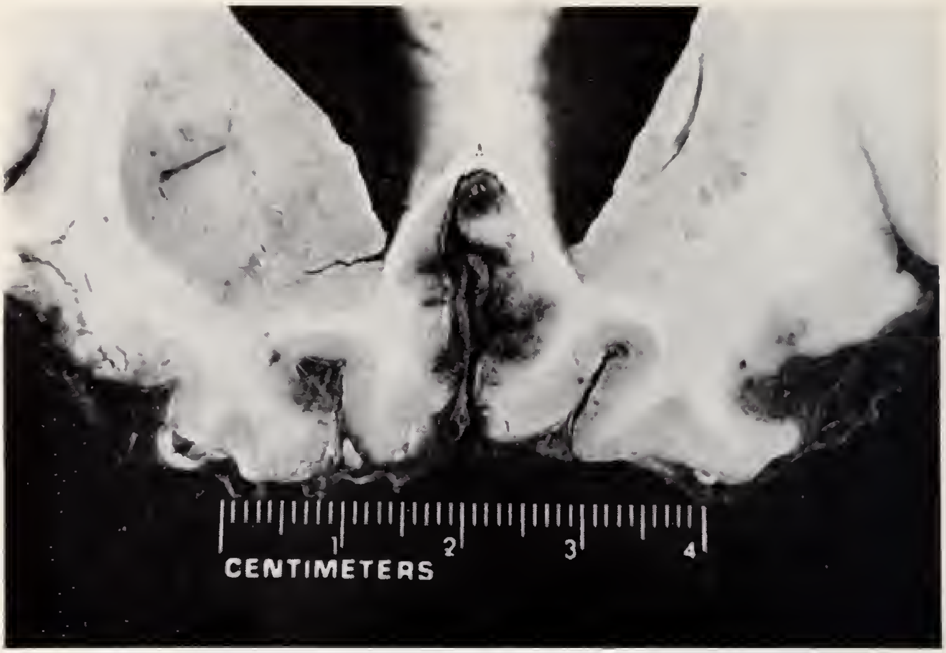

Fig. 5. Transverse section through frontal lobes in the region of the frontal horns. Cerebral cortex shows superficial invasion by pigmented neoplasm.

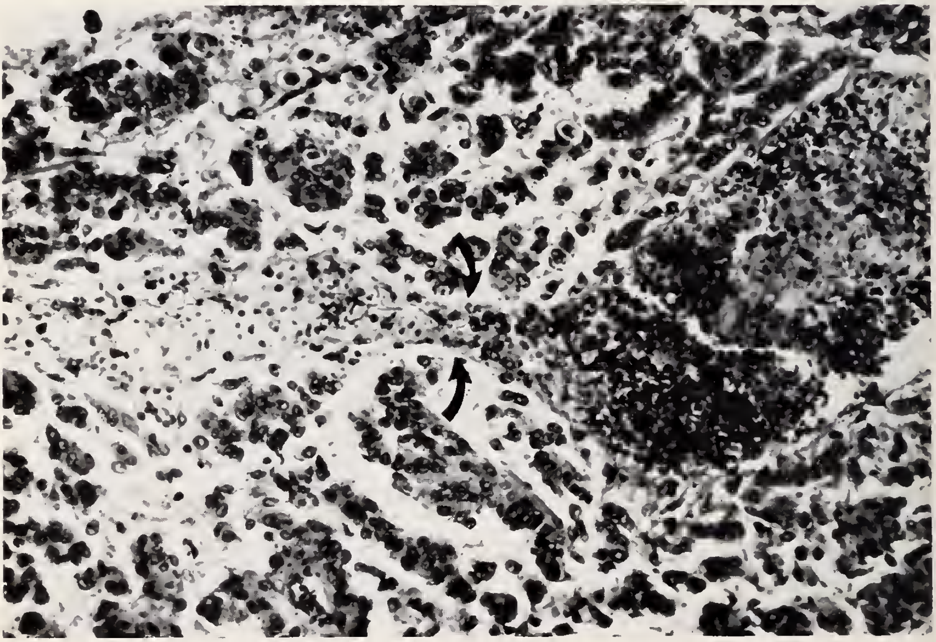

Fig. 6. Tumor cells in the leptomeninges. Note focal compression of small vein (arrows) (H&E × 291).

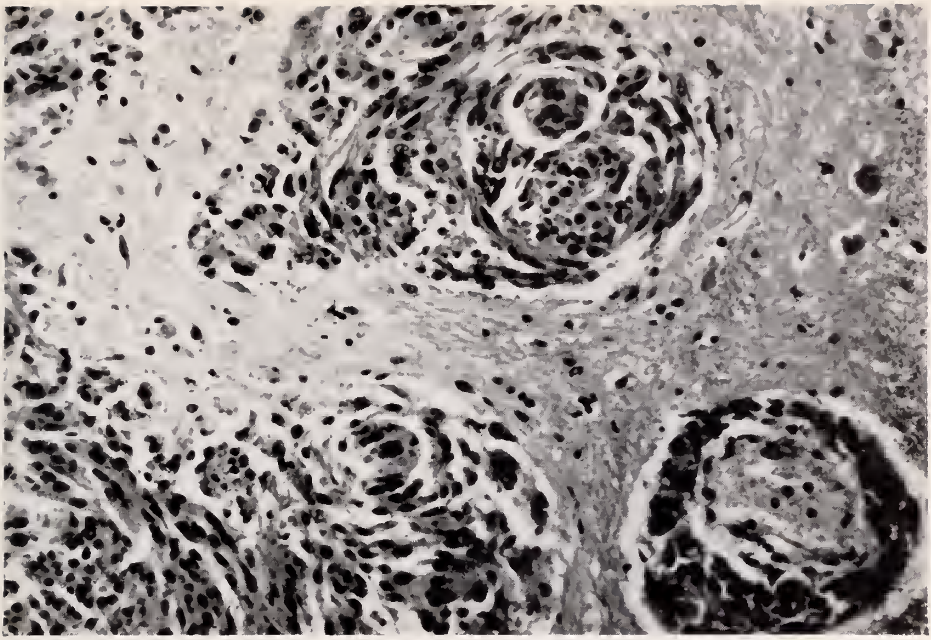

FIG. 7. Small blood vessels in cerebral cortex and subcortical white matter are invested by sheaths of tumor cells (H&E × 291).

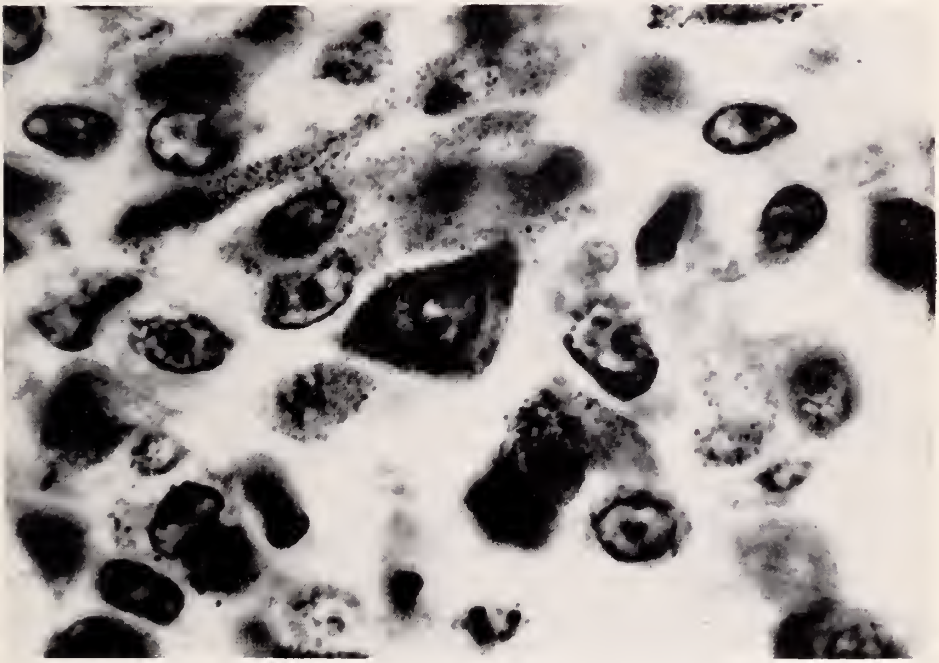

FIG. 8. Details of typical melanoma tumor cells in the leptomeninges. Nuclei have thick nuclear membranes and prominent nucleoli. Cytoplasm of most tumor cells contains melanin granules (H&E × 1848).

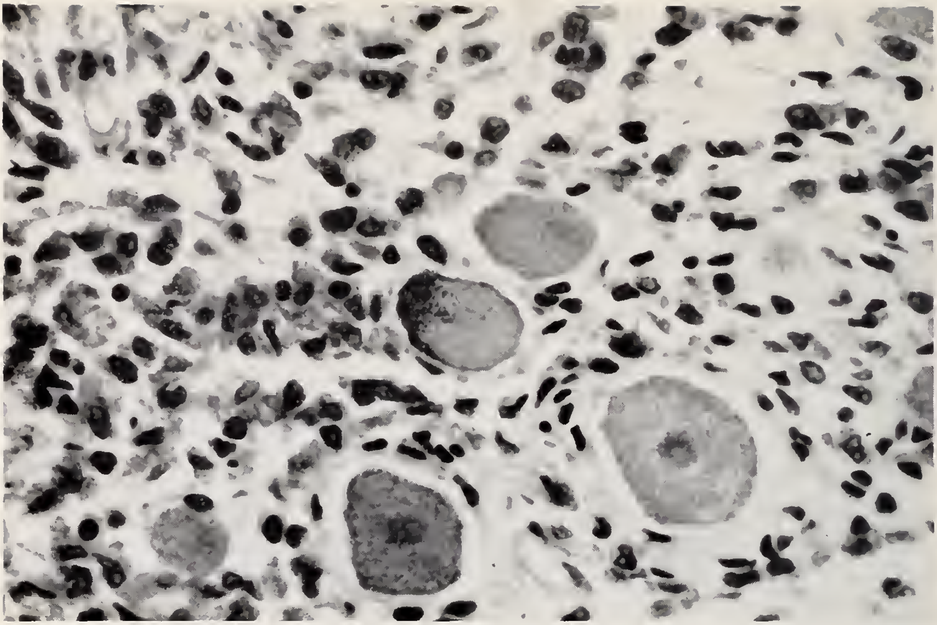

Fig. 9. Trigeminal ganglion infiltrated by tumor cells. Neurons show varying degrees of chromatolysis. Note the preservation of neuromelanin granules in the centrally situated neuron (H&E × 739).

3. malignant melanoma
4. tuberculosis
5. mycoses
6. syphilis
7. sarcoidosis
8. invasive glioblastoma
9. primary melanomatosis

Negative cultures, serology, and antibody titers as well as normal complete blood count, urinalysis, serum chemistries, and plain x-rays limited the possibilities; but a working diagnosis could only be reached by cerebrospinal fluid cytology, pneumoencephalography, angiography, and surgical biopsy. Conclusive determination was not made in our patient or in virtually all of the reported cases until postmortem examination.

Solitary metastases to the central nervous system from malignant dermal melanomas are common, and often the original site cannot be ascertained; before making the diagnosis of primary melanoma of the leptomeninges, there should be clear histologic evidence that melanocytosis preceded melanomatous transformation (16). In the present case, different areas of the neoplasm represent the full panorama of hyperplasia, dysplasia, metaplasia, and anaplasia. Winkelman, Gotten, and Silverstein (3) established the most widely accepted principles for confirming the diagnosis: the presence of a diffuse melanoblastic infiltration of the leptomeninx, and adequate necropsy including the orbital contents to verify the absence of any other source.

Complete histologic sectioning in most published autopsies revealed extensive infiltration of the subarachnoid space with perivascular cuffing and cranial nerve root involvement. Communicating hydrocephalus often resulted from blockage of the foramina of Lushka and Magendie in the cisterna magna and obliteration of the arachnoid villi over the convexity of the cerebral hemispheres. A rapidly deteriorating clinical course and death within five months after presentation were regularly observed (17).

Summary

A case of primary leptomeningeal melanomatosis in a twenty-seven year-old woman is reported. Cerebrospinal fluid cytology, neuroradiology studies, surgical biopsy, and autopsy findings are presented. The differential diagnosis and pathophysiology are discussed.

Acknowledgment

Dr. Herbert E. Nieburgs prepared and interpreted the cerebrospinal fluid cytology.

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RADIOLOGIC NOTES

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CASE NO. 385

Submitted by

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A 65-year-old man presented with a history of pain in the right leg with some backache for six months. There was no hematuria. Excretory urography was performed prior to hospital admission. A large mass arising from the lower pole of the right kidney was demonstrated. Nephrotomography revealed that the mass was relatively lucent; however, a thin wall was not demonstrated so that a diagnosis of cyst could not be made. Therefore an ultrasonogram was done.

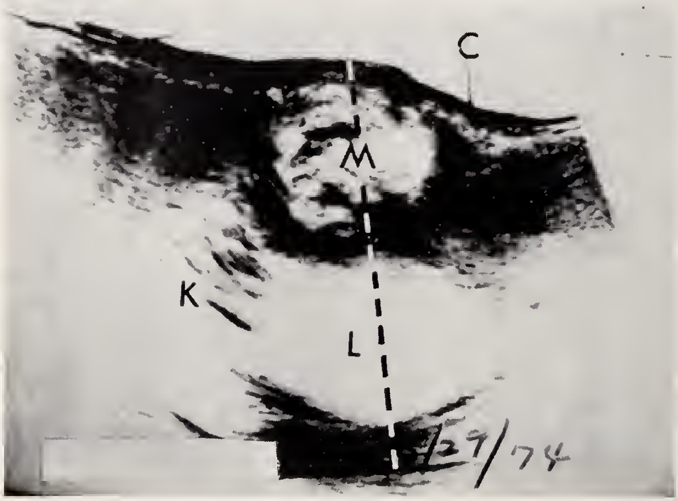

Fig. 1. Posterior longitudinal B-scan of right kidney. Left side is up. The relation between kidney, cystic mass and the solid mass is shown. Abbreviation: C = iliac crest level. M = solid mass, L = liquid containing (or cystic) mass. K = kidney.

The ultrasound B-scan* in the prone position (Fig. 1, 2) shows a large transonic lesion of 12 cm × 9.5 cm with a smooth margin in the lower pole of the right kidney. The A-mode study (Fig. 3) reveals that the lesion is free of internal echos indicating a cystic or liquid containing structure. Another lesion

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* Rohnar system from Rohé Scientific Corporation. Gray scale B-scan with 2.25 MHZ transducer.

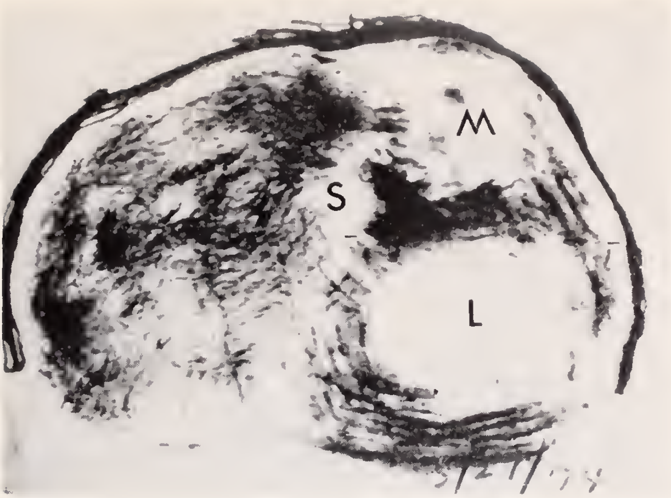

Fig. 2. Posterior transverse B-scan—Abbreviation: S = spine.

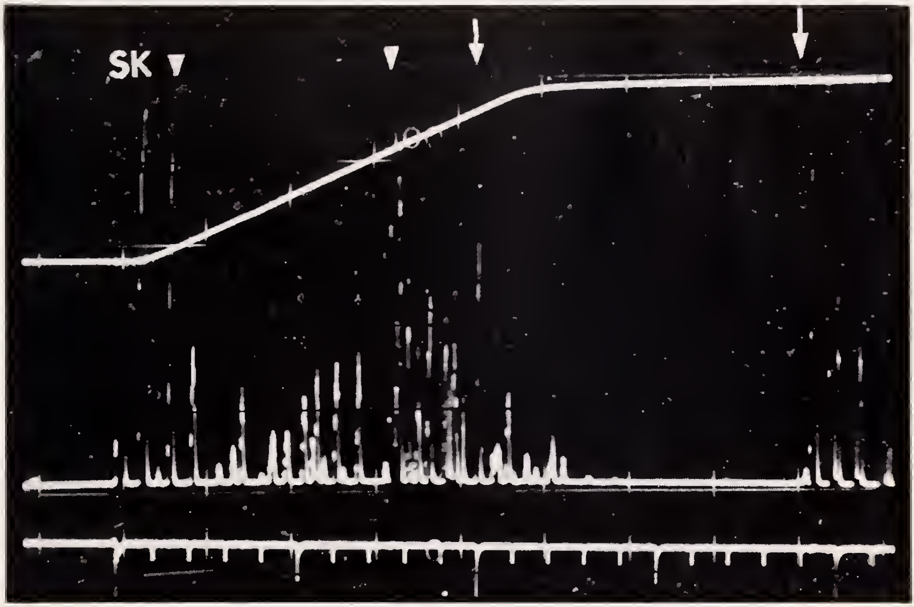

Fig. 3. A-mode tracing through the dot line on Fig. 1. The solid mass is indicated between the arrowheads. Liquid containing mass is betweeen the arrows. Abbreviation: SK = skin surface.

of 9 cm × 8 cm with multiple irregular internal echos and an irregular margin is located posterior to the right kidney and the above mentioned cystic lesion. The multiple internal echos indicate that the lesion is solid in consistency. The same areas were studied in the supine position (Figs. 4, 5). The cyst-like structure is again seen. However, a solid nodule of 2.5 cm × 4 cm is found at its inferior

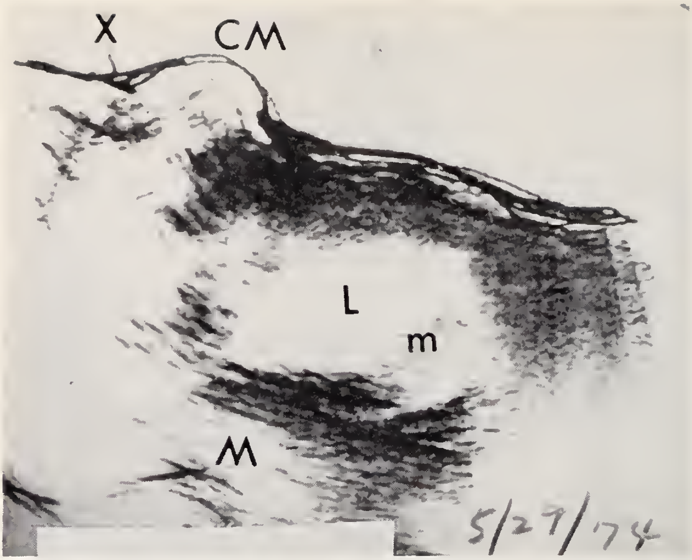

Fig. 4. Anterior longitudinal B-scan. The small solid mass is seen on the inferior aspect of the large cystic renal lesion. Abbreviation: X = xyphoid level. CM = costal margin. m = mass in wall of renal cystic lesion.

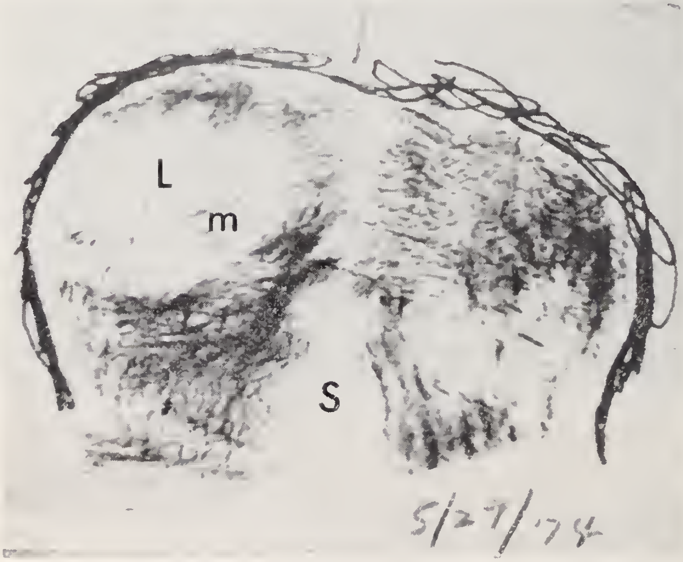

Fig. 5. Anterior transverse B-scan. The small mass (m) is seen on the postero-medial wall of the cystic renal lesion.

posteromedial wall. The ultrasonic findings indicate that the large cystic renal lesion is not a simple cyst. The possibilities are: 1) a cyst with an organized blood clot; 2) a cyst with a tumor on the wall; 3) a tumor with liquified necrosis.

The patient was, then, admitted to the hospital for angiography. Following a flush aortogram which demonstrated some abnormal vessels in the area of the mass, a selective renal arteriogram was performed. The mass arising from the lower pole of the right kidney was avascular. The capsular vessel was stretched around the periphery of the mass (Fig. 6).

Selective arteriography of the right third lumbar artery demonstrated that this vessel was enlarged (Fig. 7). In addition, the abnormal vessels seen on the aortogram were shown to be fed from the lumbar circulation. These vessels were tortous and irregular having the appearance of neovasculature.

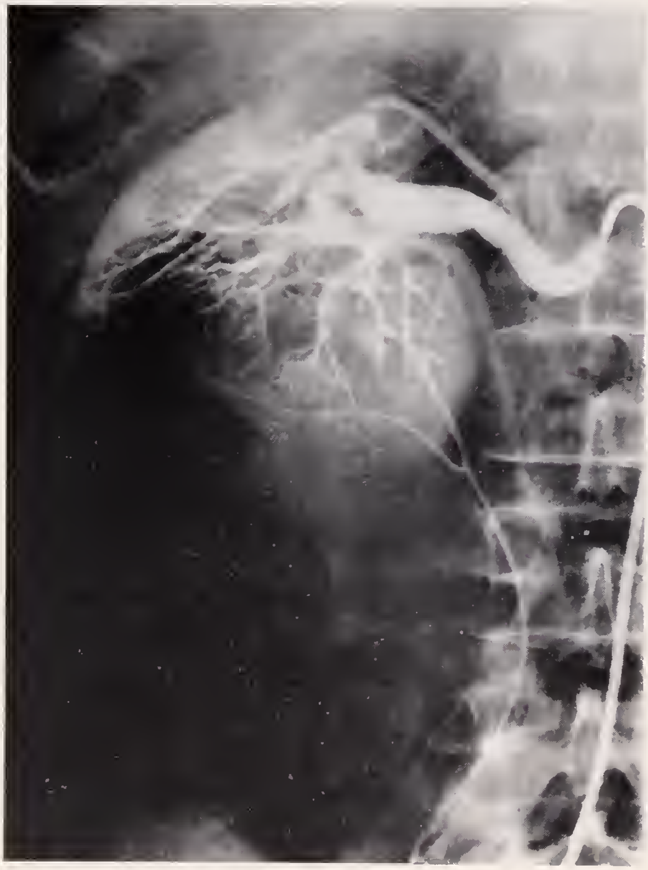

Fig. 6. An avascular mass at the lower pole of the right kidney is seen. There is displacement of a renal capsular branch around the periphery of the mass. Note destroyed vertebral pedicle at L2.

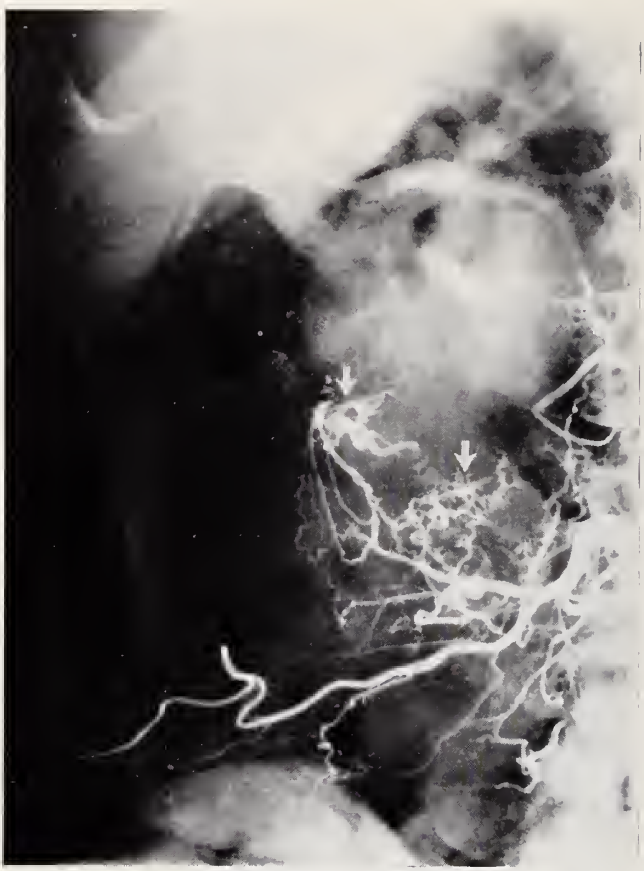

Fig. 7. Selective injection of the right third lumbar artery. This vessel is enlarged and feeds a group of tortuous irregular tumor vessels (arrows).

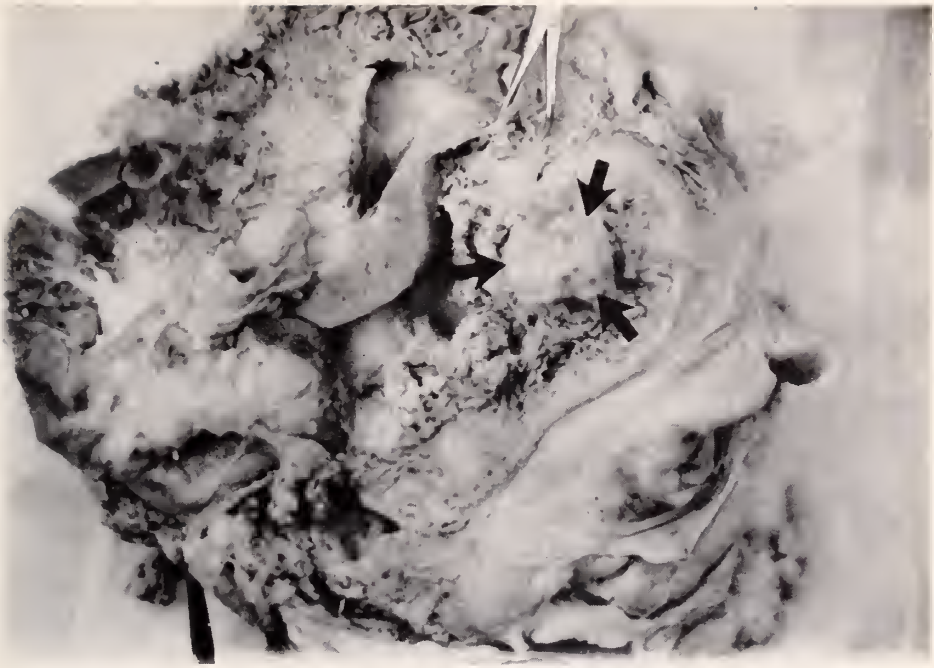

Fig. 8. Surgical specimen of right kidney and its lower pole mass. The kidney and the mass was opened from its lateral aspect. The upper pole of the kidney is on the left and the mass on the right. Arrows indicate the nodule on the inner wall of the liquefied mass.

At operation, a large tumor mass with cystic degeneration was found at the lower pole of the right kidney (Fig. 8). There was a nodule on the inner wall of the liquified mass. Another mass was removed from the muscle of the back posterior to the right kidney. Histological study revealed that the renal mass was a large papillary renal cortical carcinoma with extensive necrosis and invasion of renal capsule and the mass in the muscle was from the same origin but with different cell type, anaplastic spindle cell.

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